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(57) Abstract Disclosed are therapeutic treatment methods, compositions and devices for maintaining neural pathways in a mammal, including enhancing survival of neurons at risk of dying, inducing cellular repair of damaged neurons and neural pathways, and stimulating neurons to maintain their differentiated phenotype. In one embodiment, the invention provides means for stimulating CAM expression in neurons. The invention also provides means for evaluating the status of nerve tissue, including means for detecting and monitoring neuropathies in a mammal. The methods, devices and compositions include a morphogen-stimulating agent provided to the mammal in a therapeutically effective concentration.			

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Morphogen-Induced Nerve Regeneration and Repair

5

BACKGROUND OF THE INVENTION

The present invention relates to methods for enhancing the survival of neuronal cells in vivo and to methods, compositions and devices for maintaining neural pathways in vivo. More particularly, the invention provides methods for enhancing survival of neuronal cells at risk of dying, including methods for redifferentiating transformed cells of neural origin and methods for maintaining phenotypic expression of differentiated neuronal cells. The invention also provides means for repairing damaged neural pathways, including methods for stimulating axonal growth over extended distances, and methods for alleviating immunologically-related nerve tissue damage. In a particular embodiment of the invention, this invention provides a method for stimulating cell adhesion molecule expression in cells, and particularly nerve cell adhesion molecule expression in neurons. Finally, the invention provides means for evaluating nerve tissue stasis and identifying neural dysfunction in a mammal.

The mammalian nervous system comprises a peripheral nervous system (PNS) and a central nervous system (CNS, comprising the brain and spinal cord), and is composed of two principal classes of cells: neurons and glial cells. The glial cells fill the spaces between neurons, nourishing them and modulating their function. Certain glial cells, such as Schwann cells in the PNS

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and oligodendrocytes in the CNS, also provide a protective myelin sheath that surrounds and protects neuronal axons, which are the processes that extend from the neuron cell body and through which the
5 electric impulses of the neuron are transported. In the peripheral nervous system, the long axons of multiple neurons are bundled together to form a nerve or nerve fiber. These, in turn, may be combined into fascicles, wherein the nerve fibers form bundles
10 embedded, together with the intraneuronal vascular supply, in a loose collagenous matrix bounded by a protective multilamellar sheath. In the central nervous system, the neuron cell bodies are visually distinguishable from their myelin-ensheathed processes,
15 and are referenced in the art as grey and white matter, respectively.

During development, differentiating neurons from the central and peripheral nervous systems send out
20 axons that must grow and make contact with specific target cells. In some cases, growing axons must cover enormous distances; some grow into the periphery, whereas others stay confined within the central nervous system. In mammals, this stage of neurogenesis is
25 complete during the embryonic phase of life and neuronal cells do not multiply once they have fully differentiated.

Accordingly, the neural pathways of a mammal are
30 particularly at risk if neurons are subjected to mechanical or chemical trauma or to neuropathic degeneration sufficient to put the neurons that define the pathway at risk of dying. A host of neuropathies, some of which affect only a subpopulation or a system
35 of neurons in the peripheral or central nervous systems

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have been identified to date. The neuropathies, which may affect the neurons themselves or the associated glial cells, may result from cellular metabolic dysfunction, infection, exposure to toxic agents,

- 5 autoimmunity dysfunction, malnutrition or ischemia. In some cases the cellular dysfunction is thought to induce cell death directly. In other cases, the neuropathy may induce sufficient tissue necrosis to stimulate the body's immune/inflammatory system and the
10 mechanisms of the body's immune response to the initial neural injury then destroys the neurons and the pathway defined by these neurons.

Currently no satisfactory method exists to repair
15 the damage caused by these neuropathies, which include multiple sclerosis, amyotrophic lateral sclerosis (ALS), Huntington's chorea, Alzheimer's disease, Parkinson's disease (parkinsonism), and metabolically derived disorders, such as hepatic encephalopathy.

- 20 Current attempts to counteract the effects of severe traumatic or neural degenerative lesions of the brain and/or spinal cord have to date primarily involved implantation of embryonic neurons in an effort to replace functionally, or otherwise compensate for, lost
25 or deficient neurons. Currently, however, human fetal cell transplantation research is severely restricted. Administration of neurotrophic factors such as nerve growth factor and insulin-like growth factor also have been suggested to stimulate neuronal growth within the
30 CNS. (See, for example, Lundborg, (1987) Acta Orthop. Scand. 58:145-169 and US Pat. No. 5,093,317.) Administration of neurotrophic factors to the CNS requires bypassing the blood-brain barrier. The barrier may be overcome by direct infusion, or by
35 modifying the molecule to enhance its transport across

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the barrier, as by chemical modification or conjugation, or by molecule truncation. Schwann cells also have been grafted to a site of a CNS lesion in an attempt to stimulate and maintain growth of damaged 5 neuronal processes (Paino et al. (1991) Exp. Neurology 114(2):254-257).

Where the damaged neural pathway results from CNS axonal damage, autologous peripheral nerve grafts have 10 been used to bridge lesions in the central nervous system and to allow axons to make it back to their normal target area. In contrast to CNS neurons, neurons of the peripheral nervous system can extend new peripheral processes in response to axonal damage. This 15 regenerative property of peripheral nervous system axons is thought to be sufficient to allow grafting of these segments to CNS axons. Successful grafting appears to be limited, however, by a number of factors, including the length of the CNS axonal lesion to be 20 bypassed, and the distance of the graft sites from the CNS neuronal cell bodies, with successful grafts occurring near the cell body.

Within the peripheral nervous system, this cellular 25 regenerative property of neurons has limited ability to repair function to a damaged neural pathway. Specifically, the new axons extend randomly, and are often misdirected, making contact with inappropriate targets that can cause abnormal function. For example, 30 if a motor nerve is damaged, regrowing axons may contact the wrong muscles, resulting in paralysis. In addition, where severed nerve processes result in a gap of longer than a few millimeters, e.g., greater than 10

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millimeters (mm), appropriate nerve regeneration does not occur, either because the processes fail to grow the necessary distance, or because of misdirected axonal growth. Efforts to repair peripheral nerve

- 5 damage by surgical means has met with mixed results, particularly where damage extends over a significant distance. In some cases, the suturing steps used to obtain proper alignment of severed nerve ends stimulates the formulation of scar tissue which is
- 10 thought to inhibit axon regeneration. Even where scar tissue formation has been reduced, as with the use of nerve guidance channels or other tubular prostheses, successful regeneration generally still is limited to nerve damage of less than 10 millimeters in distance.
- 15 In addition, the reparative ability of peripheral neurons is significantly inhibited where an injury or neuropathy affects the cell body itself or results in extensive degeneration of a distal axon.
- 20 Mammalian neural pathways also are at risk due to damage caused by neoplastic lesions. Neoplasias of both the neurons and glial cells have been identified. Transformed cells of neural origin generally lose their ability to behave as normal differentiated cells and
- 25 can destroy neural pathways by loss of function. In addition, the proliferating tumors may induce lesions by distorting normal nerve tissue structure, inhibiting pathways by compressing nerves, inhibiting cerebrospinal fluid or blood supply flow, and/or by stimulating the
- 30 body's immune response. Metastatic tumors, which are a significant cause of neoplastic lesions in the brain and spinal cord, also similarly may damage neural pathways and induce neuronal cell death.

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One type of morphoregulatory molecule associated with neuronal cell growth, differentiation and development is the cell adhesion molecule ("CAM"), most notably the nerve cell adhesion molecule (N-CAM). CAMs 5 belong to the immunoglobulin super-family and mediate cell-cell interactions in developing and adult tissues through homophilic binding, i.e., CAM-CAM binding on apposing cells. A number of different CAMs currently have been identified. Of these, the most thoroughly 10 studied to date are N-CAM and L-CAM (liver cell adhesion molecules), both of which have been identified on all cells at early stages of development, as well as in different adult tissues. In neural tissue development, N-CAM expression is believed to be 15 important in tissue organization, neuronal migration, nerve-muscle tissue adhesion, retinal formation, synaptogenesis, and neural degeneration. Reduced N-CAM expression also is thought to be associated with nerve dysfunction. For example, expression of at least one 20 form of N-CAM, N-CAM-180, is reduced in a mouse dysmyelinating mutant (Bhat (1988) Brain Res. 452:373-377). Reduced levels of N-CAM also have been associated with normal pressure hydrocephalus (Werdelin 25 (1989) Acta Neurol. Scand. 79:177-181), and with type II schizophrenia (Lyons et al., (1988) Biol. Psychiatry 23:769-775.) In addition, antibodies to N-CAM have been shown to disrupt functional recovery in injured nerves (Remsen (1990) Exp. Neurobiol. 110:268-273).

30 It is an object of this invention to provide methods for enhancing survival of neurons at risk of dying in a mammal. Another object is to provide methods for maintaining neural pathways in vivo at risk of injury, or following damage to nerve tissue due to 35 mechanical or chemical trauma, a neuropathy, or a

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neoplastic lesion. Another object is to provide compositions and devices for repairing gaps in a neural pathway of the peripheral nervous system. Yet another object is to provide a means for redifferentiating
5 transformed cells defining neural pathways, particularly transformed cells of neural origin. Another object is to provide a means for stimulating CAM expression, particularly N-CAM expression in a cell. Yet another object is to provide methods for
10 monitoring the status of nerve tissue by monitoring fluctuations in protein levels present in nerve tissue, serum and/or cerebrospinal fluid. These and other objects and features of the invention will be apparent from the description, drawings, and claims
15 which follow.

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Summary of the Invention

The present invention provides methods and
5 compositions for maintaining neural pathways in a
mammal in vivo, including methods for enhancing the
survival of neural cells.

In one aspect, the invention features compositions
10 and therapeutic treatment methods that comprise the
step of administering to a mammal a therapeutically
effective amount of a morphogenic protein
("morphogen"), as defined herein, upon injury to a
neural pathway, or in anticipation of such injury, for
15 a time and at a concentration sufficient to maintain
the neural pathway, including repairing damaged
pathways, or inhibiting additional damage thereto.

In another aspect, the invention features
20 compositions and therapeutic treatment methods for
maintaining neural pathways in a mammal in vivo which
include administering to the mammal, upon injury to a
neural pathway or in anticipation of such injury, a
compound that stimulates in vivo a therapeutically
25 effective concentration of an endogenous morphogen
within the body of the mammal sufficient to maintain
the neural pathway, including repairing damaged
pathways or inhibiting additional damage thereto.
These compounds are referred to herein as morphogen-
30 stimulating agents, and are understood to include
substances which, when administered to a mammal, act on
tissue(s) or organ(s) that normally are responsible

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for, or capable of, producing a morphogen and/or secreting a morphogen, and which cause the endogenous level of the morphogen to be altered. The agent may act, for example, by stimulating expression and/or

5 secretion of an endogenous morphogen.

In particular, the invention provides methods for enhancing the survival of neurons at risk of dying, including protecting neurons from the tissue

10 destructive effects associated with the body's immune/inflammatory response to a nerve injury. The invention also provides methods for stimulating neurons to maintain their differentiated phenotype, including inducing the redifferentiation of transformed cells of
15 neuronal origin to a morphology characteristic of untransformed neurons. In one embodiment, the invention provides means for stimulating production of cell adhesion molecules in cells, particularly nerve cell adhesion molecules (N-CAM) in neurons. The
20 invention also provides methods, compositions and devices for stimulating cellular repair of damaged neurons and neural pathways, including regenerating damaged axons of the peripheral and central nervous systems. In addition, the invention also provides
25 means for evaluating the status of nerve tissue, and for detecting and monitoring neuropathies in a mammal by monitoring fluctuations in the morphogen levels or endogenous morphogen antibody levels present in a mammal's serum or cerebrospinal fluid.

30

As used herein, a "neural pathway" describes a nerve circuit for the passage of electric signals from a source to a target cell site. The pathway includes the neurons through which the electric impulse is

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transported, including groups of interconnecting neurons, the nerve fibers formed by bundled neuronal axons, and the glial cells surrounding and associated with the neurons.

5

- In one aspect of the invention, the morphogens described herein are useful in repairing damaged neural pathways of the peripheral nervous system. In particular, the morphogens are useful for repairing 10 damaged pathways, including transected or otherwise damaged nerve fibers (nerves) requiring regeneration of neuronal processes, particularly axons, over extended distances to bridge a gap in the nerve itself, or between the nerve and a post-synaptic cell.
- 15 Specifically, the morphogens described herein are capable of stimulating complete axonal nerve regeneration, including vascularization and reformation of the protective myelin sheath. The morphogen preferably is provided to the site of injury dispersed 20 in a biocompatible, bioresorbable carrier material capable of maintaining the morphogen at the site and, where necessary, means for directing axonal growth from the proximal to the distal ends of a severed neuron or nerve. For example, means for directing axonal growth 25 may be required where nerve regeneration is to be induced over an extended distance, such as greater than 10 mm. Many carriers capable of providing these functions are envisioned. For example, useful carriers include substantially insoluble materials or viscous 30 solutions prepared as disclosed herein comprising laminin, hyaluronic acid or collagen, or other suitable synthetic, biocompatible polymeric materials such as polylactic, polyglycolic or polybutyric acids and/or copolymers thereof. The currently preferred carrier 35 comprises an extracellular matrix composition, such as

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one described herein derived, for example, from mouse sarcoma cells. Also envisioned as especially useful are brain tissue-derived extracellular matrices.

- 5 In a particularly preferred embodiment, the morphogen is provided to the site as part of a device wherein the morphogen is disposed in a nerve guidance channel which spans the distance of the damaged pathway. The channel acts both as a protective
10 covering and a physical means for guiding growth of a neuronal process such as an axon. Useful channels comprise a biocompatible membrane or casing, which may be tubular in structure, having a dimension sufficient to span the gap or break in the nerve to be repaired,
15 and having openings adapted to receive severed nerve ends. The casing or membrane may be made of any biocompatible, nonirritating material, such as silicone or a biocompatible polymer such as polyethylene or polyethylene vinyl acetate. The casing also may be
20 composed of biocompatible, bioresorbable polymers, including, for example, collagen, hyaluronic acid, polylactic, polybutyric and polyglycolic acids. In a currently preferred embodiment, the outer surface of the channel is substantially impermeable.
25
The morphogen may be disposed in the channel in association with a biocompatible carrier material, or it may be adsorbed to or otherwise associated with the inner surface of the casing, such as is described in
30 U.S. Pat. No. 5,011,486, provided that the morphogen is accessible to the severed nerve ends. Additionally, although the nerve guidance channels described herein generally are tubular in shape, it should be evident to those skilled in the art that various alternative
35 shapes may be employed. The lumen of the guidance

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channels may, for example, be oval or even square in cross section. Moreover the guidance channels may be constructed of two or more parts which may be clamped together to secure the nerve stumps. Nerve endings may
5 be secured to the nerve guidance channels by means of sutures, biocompatible adhesives such as fibrin glue, or other means known in the medical art.

The morphogens described herein also are envisioned
10 to be useful in autologous peripheral nerve segment implants to bypass damaged neural pathways in the central nervous system, such as in the repair of damaged or detached retinas, or other damage to the optic nerve. Here the morphogen is provided to the

15 site of attachment to stimulate axonal growth at the graft site, particularly where the damaged axonal segment to be bypassed occurs far from the neuronal cell body.

20 The morphogens described herein also are useful for enhancing survival of neuronal cells at risk of dying, thereby preventing, limiting or otherwise inhibiting damage to neural pathways. Non-mitotic neurons are at risk of dying as a result of a neuropathy or other

25 cellular dysfunction of a neuron or glial cell inducing cell death, or following a chemical or mechanical lesion to the cell or its surrounding tissue. The chemical lesions may result from known toxic agents, including lead, ethanol, ammonia, formaldehyde and many

30 other organic solvents, as well as the toxins in cigarette smoke and opiates. Excitatory amino acids, such as glutamate also may play a role in the pathogenesis of neuronal cell death (see Freese et al. (1990) Brain Res. 521:254-264). Neuronal cell death
35 also is thought to be a significant contributing factor

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in a number of neurodegenerative diseases, including Alzheimer's disease, Huntington's chorea, and Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis. The etiology of these neuropathies 5 may be metabolic, as results in hepatic encephalopathy, infectious, toxic, autoimmune, nutritional or ischemic. In addition, ethanol and a number of other toxins also have been identified as significant contributing factors in neurodegenerative diseases. The morphogens 10 described herein may be provided to cells at risk of dying to enhance their survival and thereby protect the integrity of the neural pathway. The morphogens may be provided directly to the site, or they may be provided systemically. Alternatively, as described above, an 15 agent capable of stimulating endogenous morphogen expression and/or secretion, preferably in cells associated with the nerve tissue of interest, may be administered to the mammal.

20 In another aspect of the invention, the method disclosed is useful for redifferentiating transformed cells, particularly transformed cells of neuronal or glial origin, such that the morphogen-treated cells are induced to display a morphology characteristic of 25 untransformed cells. Where the transformed cells are cells of neuronal origin, morphogen treatment preferably induces cell rounding and cell aggregation (clumping), cell-cell adhesion, neurite outgrowth formation and elongation, and N-CAM production. The 30 methods described herein are anticipated to substantially inhibit or reduce neural cell tumor formation and/or proliferation in nerve tissue. It is anticipated that the methods of this invention will be useful in substantially reducing the effects of various 35 carcinomas of nerve tissue origin such as

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retinoblastomas, neuroblastomas, and gliomas or glioblastomas. In addition, the method also is anticipated to aid in inhibiting neoplastic lesions caused by metastatic tissue. Metastatic tumors are one 5 of the most common neoplasms of the CNS, as they can reach the intracranial compartment through the bloodstream. Metastatic tumors may damage neural pathways for example, by distorting normal nerve tissue structure, compressing nerves, blocking flow of 10 cerebrospinal fluid or the blood supply nourishing brain tissue, and/or by stimulating the body's immune response.

In another aspect of the invention, the morphogens 15 described herein are useful for providing neuroprotective effects to alleviate neural pathway damage associated with the body's immune/inflammatory response to an initial injury to nerve tissue. Such a response may follow trauma to nerve tissue, caused, for 20 example, by an autoimmune dysfunction, neoplastic lesion, infection, chemical or mechanical trauma, disease, by interruption of blood flow to the neurons or glial cells, for example following ischemia or hypoxia, or by other trauma to the nerve or surrounding 25 material. For example, the primary damage resulting from hypoxia or ischemia-reperfusion following occlusion of a neural blood supply, as in an embolic stroke, is believed to be immunologically associated. In addition, at least part of the damage associated 30 with a number of primary brain tumors also appears to be immunologically related. Application of the morphogen directly to the cells to be treated, or providing the morphogen to the mammal systemically, for example, intravenously or indirectly by oral 35 administration, may be used to alleviate and/or inhibit

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the immunologically related response to a neural injury. Alternatively, administration of an agent capable of stimulating morphogen expression and/or secretion in vivo, preferably at the site of injury,

5 also may be used. Where the injury is to be induced, as during surgery or other aggressive clinical treatment, the morphogen or agent may be provided prior to induction of the injury to provide a neuroprotective effect to the nerve tissue at risk.

10

In still another aspect, the invention described herein provides methods for supporting the growth and maintenance of differentiated neurons, including inducing neurons to continue expressing their

15 phenotype. It is anticipated that this activity will be particularly useful in the treatment of nerve tissue disorders where loss of function is caused by reduced or lost cellular metabolic function and cells become senescent or quiescent, such as is thought to occur in
20 aging cells and to be manifested in Alzheimer's disease. Application of the morphogen directly to cells to be treated, or providing it systemically by parenteral or oral administration stimulates these cells to continue expressing their phenotype,

25 significantly inhibiting and/or reversing the effects of the cellular metabolic dysfunction, thereby maintaining the neural pathway at risk. Alternatively, administration of an agent capable of stimulating endogenous morphogen expression and/or secretion in
30 vivo may be used.

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In still another aspect, the invention provides methods for stimulating CAM expression levels in a cell, particularly N-CAM expression in neurons. CAMs are molecules defined as carrying out cell-cell interactions necessary for tissue formation. CAMs are believed to play a fundamental regulatory role in tissue development, including tissue boundary formation, embryonic induction and migration, and tissue stabilization and regeneration. Altered CAM levels have been implicated in a number of tissue disorders, including congenital defects, neoplasias, and degenerative diseases.

In particular, N-CAM expression is associated with normal neuronal cell development and differentiation, including retinal formation, synaptogenesis, and nerve-muscle tissue adhesion. Inhibition of one or more of the N-CAM isoforms is known to prevent proper tissue development. Altered N-CAM expression levels also are associated with neoplasias, including neuroblastomas (see infra), as well as with a number of neuropathies, including normal pressure hydrocephalus and type II schizophrenia. Application of the morphogen directly to the cells to be treated, or providing the morphogen to the mammal systemically, for example, parenterally, or indirectly by oral administration, may be used to induce cellular expression of one or more CAMs, particularly N-CAMs. Alternatively, administration of an agent capable of stimulating morphogen expression and/or secretion in vivo, preferably at the site of injury, also may be used to induce CAM production.

CAMs also have been postulated as part of a morphoregulatory pathway whose activity is induced by a to date unidentified molecule (See, for example,

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Edelman, G.M. (1986) Ann. Rev. Cell Biol. 2:81-116). Without being limited to any given theory, the morphogens described herein may act as the inducer of this pathway.

5

Finally, modulations of endogenous morphogen levels may be monitored as part of a method of detecting nerve tissue dysfunction. Specifically, modulations in endogenous morphogen levels are anticipated to reflect 10 changes in nerve tissue status. Morphogen expression may be monitored directly in biopsied cell samples, in cerebrospinal fluid, or serum. Alternatively, morphogen levels may be assessed by detecting changes in the levels of endogenous antibodies to the 15 morphogen. For example, one may obtain serum samples from a mammal, and then detect the concentration of morphogen or antibody present in the fluid by standard protein detection means known to those skilled in the art. As an example, binding protein capable of 20 interacting specifically with the morphogen of interest such as an anti-morphogen antibody may be used to detect a morphogen in a standard immunoassay. The morphogen levels detected then may be compared to a previously determined standard or reference level, with 25 changes in the detected levels being indicative of the status of the tissue.

In one preferred embodiment of the invention, the morphogen or morphogen-stimulating agent is 30 administered systemically to the individual, e.g., orally or parenterally. In another embodiment of the invention, the morphogen may be provided directly to the nerve tissue, e.g., by injection to the cerebral spinal fluid or to a nerve tissue locus.

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In any treatment method of the invention, "administration of morphogen" refers to the administration of the morphogen, either alone or in combination with other molecules. For example, the 5 mature form of the morphogen may be provided in association with its precursor "pro" domain, which is known to enhance the solubility of the protein. Other useful molecules known to enhance protein solubility include casein and other milk components, as well as 10 various serum proteins. Additional useful molecules which may be associated with the morphogen or morphogen-stimulating agent include tissue targeting molecules capable of directing the morphogen or morphogen-stimulating agent to nerve tissue. Tissue 15 targeting molecules envisioned to be useful in the treatment protocols of this invention include antibodies, antibody fragments or other binding proteins which interact specifically with surface molecules on nerve tissue cells.

20

Still another useful tissue targeting molecule is part or all of the morphogen precursor "pro" domain, particularly that of OP-1 or GDF-1. These proteins are found naturally associated with nerve tissue but also 25 may be synthesized in other tissues and targeted to nerve tissue after secretion from the synthesizing tissue. For example, while the protein has been shown to be active in bone tissue, the primary source of OP-1 synthesis appears to be the tissue of the urogenic 30 system (e.g., renal and bladder tissue), with secondary expression levels occurring in the brain, heart and lungs (see below.) Moreover, the protein has been identified in serum, saliva and various milk forms. In addition, the secreted form of the protein comprises 35 the mature dimer in association with the pro domain of

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the intact morphogen sequence. Accordingly, the associated morphogen pro domains may act to target specific morphogens to different tissues in vivo.

5 Associated tissue targeting or solubility-enhancing molecules also may be covalently linked to the morphogen using standard chemical means, including acid-labile linkages, which likely will be preferentially cleaved in the acidic environment of
10 bone remodeling sites.

Finally, the morphogens or morphogen-stimulating agents provided herein also may be administered in combination with other molecules known to be beneficial
15 in maintaining neural pathways, including, for example, nerve growth factors and anti-inflammatory agents.

Where the morphogen is intended for use as a therapeutic for disorders of the CNS, an additional
20 problem must be addressed: overcoming the so-called "blood-brain barrier", the brain capillary wall structure that effectively screens out all but selected categories of molecules present in the blood, preventing their passage into the brain. The
25 blood-brain barrier may be bypassed effectively by direct infusion of the morphogen or morphogen-stimulating agent into the brain. Alternatively, the morphogen or morphogen-stimulating agent may be modified to enhance its transport across the
30 blood-brain barrier. For example, truncated forms of the morphogen or a morphogen-stimulating agent may be most successful. Alternatively, the morphogen or

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morphogen-stimulating agent may be modified to render it more lipophilic, or it may be conjugated to another molecule which is naturally transported across the barrier, using standard means known to those skilled in 5 the art, as, for example, described in Pardridge, Endocrine Reviews 7:314-330 (1986) and U.S. Pat. No. 4,801,575.

Accordingly, as used herein, a functional "analog" 10 of a morphogen refers to a protein having morphogenic biological activity but possessing additional structural differences compared to a morphogen as defined herein, e.g., having additional chemical moieties not normally a part of a morphogen. Such 15 moieties (introduced, for example, by acylation, alkylation, cationization, or glycosylation reactions, or other means for conjugating the moiety to the morphogen) may improve the molecule's solubility, absorption, biological half-life, or transport, e.g., 20 across the blood-brain barrier.

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as 25 well as amino acid sequence-related proteins such as DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are presented in Table II and Seq. ID Nos.5-14), 30 and the recently identified 60A protein (from *Drosophila*, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF- β super-family of proteins, share substantial amino acid sequence homology in their 35 C-terminal regions. The proteins are translated as a

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precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The signal peptide is cleaved rapidly
5 upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.)
Table I, below, describes the various morphogens identified to date, including their nomenclature as
10 used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

15

TABLE I

"OP-1" Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature,

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morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

- 5 "OP-2" refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.). The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP2). (Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.)
- 10
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- 20
- 25
- 30 "CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA
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- 23 -

5 ("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

10

15 "DPP(fx)" refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.

20

25 "Vgl(fx)" refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51: 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

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- 24 -

- 5 "Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) PNAS 86: 4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.
- 10 "GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 32. The prodomain likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372.
- 15 "60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the Drosophila 60A gene) encoding the 60A proteins (see Seq. ID No. 24 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.)
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The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.

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"BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.

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"BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27).

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25

The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.

30

"BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full length protein appear sin Celeste, et al.

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(1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

5

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention. Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain disulfide bonds such that the protein is capable of

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- 27 -

acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of 5 progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. In addition, it is also anticipated that these morphogens are capable 10 of inducing redifferentiation of committed cells under appropriate environmental conditions.

In one preferred aspect, the morphogens of this invention comprise one of two species of generic 15 amino acid sequences: Generic Sequence 1 (Seq. ID No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof. Generic Sequence 1 comprises the conserved six cysteine 20 skeleton and Generic Sequence 2 comprises the conserved six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further comprise the following additional sequence at their N- 25 terminus:

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
1 5

30 Preferred amino acid sequences within the foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4), Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31), listed below. These

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Generic Sequences accommodate the homologies shared among the various preferred members of this morphogen family identified in Table II, as well as the amino acid sequence variation among them. Specifically,

- 5 Generic Sequences 3 and 4 are composite amino acid sequences of the following proteins presented in Table II and identified in Seq. ID Nos. 5-14: human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14.) The generic sequences include both
- 10 the amino acid identity shared by the sequences in Table II, as well as alternative residues for the variable positions within the sequence. Note that these generic sequences allow for an additional cysteine at position 41 or 46 in Generic Sequences 3 or
- 15 4, respectively, providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids which influence the tertiary structure of the proteins.
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Generic Sequence 3

Leu Tyr Val Xaa Phe

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Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

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Xaa Ala Pro Xaa Gly Xaa Xaa Ala

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Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
35
5 Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
10 55 60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
15 Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Gly Cys Xaa
20 95

wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn); Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 =

- 30 -

(Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 =
5 (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at
10 res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at
15 res.49 = (Val or Met); Xaa at res.50 = (His or Asn); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser); Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56 =
20 (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg
25 or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr
30 or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 =
35 (Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His);

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Xaa at res.87 = (Arg, Gln or Glu); Xaa at res.88 =
(Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala);
Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at
res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or
5 Arg);

Generic Sequence 4

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Tyr	Val	Xaa	Phe
10	1				5				10	
	Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa	
					15					
	Xaa	Ala	Pro	Xaa	Gly	Xaa	Xaa	Ala		
		20				25				
15	Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa		
			30				35			
	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa			
					40					
	Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa		
20				45			50			
	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Xaa		
					55					
	Xaa	Cys								
			60			65				
25	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa		
				70						
	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa		
				75		80				
	Xaa	Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa		
30					85					
	Xaa	Xaa	Xaa	Xaa	Met	Xaa	Val	Xaa		
				90		95				
	Xaa	Cys	Gly	Cys	Xaa					
				100						

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wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 = (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg, or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln); Xaa at res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 = (Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp);

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Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 =
5 (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at
res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe);
Xaa at res.80 = (Phe, Tyr or Lèu); Xaa at res.81 = (Asp
or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at
res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser,
Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys);
Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or
Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at
10 res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or
Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95
= (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val,
Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa
at res.102 = (His or Arg).

15

Similarly, Generic Sequence 5 (Seq. ID No. 30) and
Generic Sequence 6 (Seq. ID No. 31) accommodate the
homologies shared among all the morphogen protein
family members identified in Table II. Specifically,
20 Generic Sequences 5 and 6 are composite amino acid
sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-
17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19),
human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22),
CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP
25 (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus,
Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13),
and GDF-1 (from mouse, Seq. ID No. 14), human BMP3
(Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human
BMP6 (Seq. ID No. 28) and 60(A) (from Drosophila, Seq.
30 ID Nos. 24-25). The generic sequences include both the
amino acid identity shared by these sequences in the
C-terminal domain, defined by the six and seven
cysteine skeltons (Generic Sequences 5 and 6,
respectively), as well as alternative residues for the
35 variable positions within the sequence. As for Generic

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Sequences 3 and 4, Generic Sequences 5 and 6 allow for an additional cysteine at position 41 (Generic Sequence 5) or position 46 (Generic Sequence 6), providing an appropriate cysteine skeleton where inter- or
5 intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

Generic Sequence 5

10

Leu Xaa Xaa Xaa Phe

1 5

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

10

15 Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala

15 20

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa

25 30

Xaa Pro Xaa Xaa Xaa Xaa Xaa

20 35

Xaa Xaa Xaa Asn His Ala Xaa Xaa

40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

50

25 Xaa Xaa Xaa Xaa Xaa Xaa Cys

55 60

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa

65

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Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
5 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Xaa Cys Xaa
95

wherein each Xaa is independently selected from a group
10 of one or more specified amino acids defined as
follows: "Res." means "residue" and Xaa at res.2 =
(Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4 =
(Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys
or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8
15 = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp,
His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or
Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 =
(Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18
= (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 =
20 (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at
res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly);
Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 =
(Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu,
Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro,
25 Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at
res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp,
Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu,
Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or
Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at
30 res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser,
Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at
res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu

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or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val);
5 Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 =
10 (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68
15 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or
20 Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln,
25 His or Val); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly
30 or Ala) and Xaa at res.97 = (His or Arg).

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Generic Sequence 6

	Cys Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe		
	1	5	10
5	Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa		
		15	
	Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala		
	20	25	
	Xaa Tyr Cys Xaa Gly Xaa Cys Xaa		
10		30	35
	Xaa Pro Xaa Xaa Xaa Xaa Xaa		
		40	
	Xaa Xaa Xaa Asn His Ala Xaa Xaa		
	45	50	
15	Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa		
		55	
	Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys		
	60	65	
	Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa		
20		70	
	Xaa Xaa Xaa Leu Xaa Xaa Xaa		
	75	80	
	Xaa Xaa Xaa Xaa Val Xaa Leu Xaa		
		85	
25	Xaa Xaa Xaa Xaa Met Xaa Val Xaa		
	90	95	
	Xaa Cys Xaa Cys Xaa		
		100	

30 wherein each Xaa is independently selected from a group
of one or more specified amino acids as defined by the
following: "Res." means "residue" and Xaa at res.2 =
(Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or
Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 =
35 (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at

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res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa
at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg,
Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or
Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16
5 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 =
(Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val);
Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or
Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg);
Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or
10 Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln,
Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at
res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at
res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 =
(Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu
15 or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at
res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 =
(Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr,
Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly
or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at
20 res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr,
Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at
res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or
Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53
25 = (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at
res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu,
Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn,
Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu,
Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at
res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 =
30 (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His);
Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro
or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at
res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val);
Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 =
35 (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or

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Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr, Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile, Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser); Xaa at res.100 = (Gly or Ala); and Xaa at res.102 = (His or Arg).

Particularly useful sequences for use as morphogens in this invention include the C-terminal domains, e.g., the C-terminal 96-102 amino acid residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A, CBMP-2B, GDF-1 (see Table II, below, and Seq. ID Nos. 5-14), as well as proteins comprising the C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see Seq. ID Nos. 24-28), all of which include at least the conserved six or seven cysteine skeleton. In addition, biosynthetic constructs designed from the generic sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S. Pat. No. 5,011,691, also are useful. Other sequences include the inhibins/activin proteins (see, for example, U.S. Pat. Nos. 4,968,590 and 5,011,691). Accordingly, other useful proteins are those exhibiting morphogenic activity and having amino acid sequences sharing at least 70% amino acid sequence homology or

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"similarity", and preferably 80% homology or similarity with any of the sequences above. These are anticipated to include allelic variants, species variants and other sequence variants (e.g., "muteins" or "mutant proteins"), whether naturally occurring or biosynthetically produced, as well as novel members of this morphogenic family of proteins.

As used herein, "amino acid sequence homology" is understood to mean amino acid sequence similarity, and homologous sequences share identical or similar amino acids, where similar amino acids are conserved amino acids as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol.5, Suppl.3, pp.345-362 (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 70% amino acid homology with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 70% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two aligned sequences. Thus, a candidate sequence sharing 60% amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence.

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As used herein, all homologies and identities calculated use OP-1 as the reference sequence. Also as used herein, sequences are aligned for homology and identity calculations using the method of Needleman et al. (1970) J.Mol. Biol. 48:443-453 and identities calculated by the Align program (DNASTar, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation.

10

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in another preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 29).

In still another preferred aspect of the invention, useful morphogens include active proteins comprising polypeptide chains encoded by nucleic acids which hybridize to DNA or RNA sequences encoding the C-terminal sequence defining the consumed cysteine domain, e.g., nucleotides 1036-1341 and nucleotides 1390-1695 of Seq. Id. Nos. 16 and 20, respectively, of OP1 or OP2 under stringent hybridization conditions. As used herein, stringent hybridization conditions are

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defined as hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

- 5 The morphogens useful in the methods, composition and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other
- 10 synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including
- 15 those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the
- 20 specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of
- 25 native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

- The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in
- 30 procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells. A detailed
 - 35 description of the morphogens useful in the methods,

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compositions and devices of this invention is disclosed in copending US patent application Serial Nos. 752,764, filed August 30, 1991, and 667,274, filed March 11, 1991, the disclosure of which are incorporated herein
5 by reference.

Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode
10 appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both prokaryotes and eukaryotes, to produce large quantities of active
15 proteins capable of maintaining neural pathways in a mammal, including enhancing the survival of neurons at risk of dying and stimulating nerve regeneration and repair in a variety of mammals, including humans.

The foregoing and other objects, features and
20 advantages of the present invention will be made more apparent from the following detailed description of the invention.

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Brief Description of the Drawings:

The foregoing and other objects and features of
this invention, as well as the invention itself, may be
5 more fully understood from the following description,
when read together with the accompanying drawings, in
which:

Fig. 1(A and B) are photomicrographs illustrating
10 the ability of morphogen (OP-1) to induce transformed
neuroblastoma x glioma cells (1A) to redifferentiate to
a morphology characteristic of untransformed neurons
(1B);

15 Fig. 2A is a dose response curve for the induction
of the 180 kDa and 140 kDa N-CAM isoforms in morphogen-
treated NG108-15 cells;

Fig. 2B is a photomicrograph of a Western blot of
20 whole cell extracts from morphogen-treated NG108-15
cells with an N-CAM-specific antibody; and

Fig. 3 is a plot of the mean number of cell
aggregates counted in 20 randomly selected fields as a
25 function of morphogen concentration.

Fig. 4 is a photomicrograph of an immunoblot
demonstrating the presence of OP-1 in human serum.

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Detailed Description of the Invention

It now has been discovered that the proteins described herein are effective agents for enhancing the survival of neurons, particularly neurons at risk of dying, and for maintaining neural pathways in a mammal. As described herein, these proteins ("morphogens") are capable of enhancing survival of non-mitotic neurons, stimulating neuronal CAM expression, maintaining the phenotypic expression of differentiated neurons, inducing the redifferentiation of transformed cells of neural origin, and stimulating axonal growth over breaks in neural processes, particularly large gaps in distal axons. The proteins also are capable of providing a neuroprotective effect to alleviate the tissue destructive effects associated with immunologically-related nerve tissue damage. Finally, the proteins may be used as part of a method for monitoring the viability of nerve tissue in a mammal.

20

Provided below are detailed descriptions of suitable morphogens useful in the methods, compositions and devices of this invention, as well as methods for their administration and application, and numerous, 25 nonlimiting examples which 1) illustrate the suitability of the morphogens and morphogen-stimulating agents described herein as therapeutic agents for maintaining neural pathways in a mammal and enhancing survival of neuronal cells at risk of dying; and 30 2) provide assays with which to test candidate morphogens and morphogen-stimulating agents for their efficacy.

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I. Useful Morphogens

As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of

5 cellular and molecular events that culminate in the formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton or its functional equivalent (see *supra*).

Specifically, the morphogens generally are capable of

10 all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting

15 the growth and maintenance of differentiated cells.

Details of how the morphogens useful in the method of this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in international

20 application US92/01968 (WO92/15323), the disclosure of which is hereby incorporated by reference. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from prokaryotic or eucaryotic host cells, using the

25 genetic sequences disclosed therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

Particularly useful proteins include those which

30 comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic constructs such as those disclosed in U.S. Pat.

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5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

5 Accordingly, the morphogens useful in the methods and compositions of this invention also may be described by morphogenically active proteins having amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with any of the sequences
10 described above, where "homology" is as defined herein above.

15 The morphogens useful in the method of this invention also can be described by any of the 6 generic sequences described herein (Generic Sequences 1, 2, 3, 4, 5 and 6). Generic sequences 1 and 2 also may include, at their N-terminus, the sequence

20 Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
 1 5

Table II, set forth below, compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human
25 OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, 30 Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), GDF-1 (from mouse, Seq. ID Nos. 14, 32 and 33), 60A protein (from Drosophila, Seq. ID Nos. 24 and 25), BMP5 (Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The sequences are aligned essentially following the method
35 of Needleman et al. (1970) J. Mol. Biol., 48:443-453,

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calculated using the Align Program (DNAstar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in 5 that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then 10 comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

TABLE II

15

	hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
	mOP-1
	hOP-2	...	Arg	Arg
	mOP-2	...	Arg	Arg
20	DPP	...	Arg	Arg	...	Ser
	Vg1	Lys	Arg	His
	Vgr-1	Gly
	CBMP-2A	Arg	...	Pro
	CBMP-2B	...	Arg	Arg	...	Ser
25	BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...
	GDF-1	...	Arg	Ala	Arg	Arg
	60A	...	Gln	Met	Glu	Thr
	BMP5
	BMP6	...	Arg
30			1				5		

	hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
	mOP-1
35	hOP-2	Gln	Leu	...

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	mOP-2	Ser	Leu	...
	DPP	Asp	...	Ser	...	Val	Asp	...
	Vgl	Glu	...	Lys	...	Val	Asn
	Vgr-1	Gln	...	Val
5	CBMP-2A	Asp	...	Ser	...	Val	Asn	...
	CBMP-2B	Asp	...	Ser	...	Val	Asn	...
	BMP3	Asp	...	Ala	...	Ile	Ser	Glu
	GDF-1	Glu	Val	His	Arg
	60A	Asp	...	Lys	His	...
10	BMP5
	BMP6	Gln
			10					15		
15	hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
	mOP-1
	hOP-2	...	Val	Gln	Ser
	mOP-2	...	Val	Gln	Ser
	DPP	Val	Leu	Asp
	Vgl	...	Val	Gln	Met
	Vgr-1	Lys
	CBMP-2A	Val	Pro	His
	CBMP-2B	Val	Pro	Gln
20	BMP3	Ser	...	Lys	Ser	Phe	Asp
	GDF-1	...	Val	Arg	...	Phe	Leu
	60A	Gly
	BMP5
25	BMP6	Lys
			20					25		
30	hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	mOP-1
	hOP-2	Ser
	mOP-2
	DPP	His	...	Lys	...	Pro

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	CBMP-2B
	BMP3	Ser	Thr	Ile	...	Ser	Ile	
	GDF-1	Leu	Val	Leu	Arg	Ala	...	
	60A	
5	BMP5	
	BMP6	
				45				50			

10	hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val	
	mOP-1	Asp	
	hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...	
	mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...	
	DPP	...	Asn	Asn	Asn	Gly	Lys	...	
15	Vgl	Ser	...	Glu	Asp	Ile	
	Vgr-1	Val	Met	Tyr	...	
	CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile	
	CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile	
	BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile	
20	GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala	
	60A	Leu	Leu	Glu	...	Lys	Lys	...	
	BMP5	Leu	Met	Phe	...	Asp	His	...	
	BMP6	Leu	Met	Tyr	...	
				55				60			

25

	hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	
	mOP-1	
	hOP-2	Ala	Lys	
30	mOP-2	Ala	Lys	
	DPP	Ala	Val	
	Vgl	...	Leu	Val	Lys	
	Vgr-1	Lys	
	CBMP-2A	Ala	Val	Glu	
35	CBMP-2B	Ala	Val	Glu	

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	BMP3	...	Glu	Val	...	Glu	Lys
	GDF-1	Asp	Leu	Val	...	Ala	Arg
	60A	Arg
	BMP5	Lys
5	BMP6	Lys
						65				70
	hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	mOP-1
10	hOP-2	...	Ser	...	Thr	Tyr
	mOP-2	...	Ser	...	Thr	Tyr
	Vgl	Met	Ser	Pro	Met	...	Phe	Tyr
	Vgr-1	Val
	DPP	...	Asp	Ser	Val	Ala	Met	Leu
15	CBMP-2A	...	Ser	Met	Leu
	CBMP-2B	...	Ser	Met	Leu
	BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
	GDF-1	...	Ser	Pro	Phe	...
	60A	...	Gly	...	Leu	Pro	His
20	BMP5
	BMP6
						75				80
	hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
25	mOP-1
	hOP-2	...	Ser	...	Asn	Arg
	mOP-2	...	Ser	...	Asn	Arg
	DPP	Asn	...	Gln	...	Thr	...	Val
	Vgl	...	Asn	Asn	Asp	Val	...	Arg
30	Vgr-1	Asn
	CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val
	CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val
	BMP3	...	Glu	Asn	Lys	Val

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	GDF-1	...	Asn	...	Asp	Val	...	Arg
	60A	Leu	Asn	Asp	Glu	Asn
	BMP5
	BMP6	Asn
5							85			
	hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	
	mOP-1
10	hOP-2	...	His	Lys	
	mOP-2	...	His	Lys	
	DPP	Asn	...	Gln	Glu	...	Thr	...	Val	
	Vg1	His	...	Glu	Ala	...	Asp	
	Vgr-1
15	CBMP-2A	Asn	...	Gln	Asp	Glu	
	CBMP-2B	Asn	...	Gln	Glu	Glu	
	BMP3	Val	...	Pro	Thr	...	Glu	
	GDF-1	Gln	...	Glu	Asp	Asp	
	60A	Ile	...	Lys	
20	BMP5	
	BMP6	Trp	
		90					95			
25	hOP-1	Ala	Cys	Gly	Cys	His				
	mOP-1				
	hOP-2				
	mOP-2				
	DPP	Gly	Arg				
30	Vg1	Glu	Arg				
	Vgr-1				
	CBMP-2A	Gly	Arg				
	CBMP-2B	Gly	Arg				
	BMP3	Ser	...	Ala	...	Arg				
35	GDF-1	Glu	Arg				

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60A	Ser
BMP5	Ser
BMP6

100

5

**Between residues 56 and 57 of BMP3 is a Val residue;
between residues 43 and 44 of GDF-1 lies
the amino acid sequence Gly-Gly-Pro-Pro.

10

As is apparent from the foregoing amino acid sequence comparisons, significant amino acid changes can be made within the generic sequences while retaining the morphogenic activity. For example, while

- 15 15 the GDF-1 protein sequence depicted in Table II shares only about 50% amino acid identity with the hOP1 sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP1 sequence, where "homology" 20 or "similarity" includes allowed conservative amino acid changes within the sequence as defined by Dayoff, et al., Atlas of Protein Sequence and Structure vol.5, supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C. 1979.)

25

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence

- 30 30 defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein. Accordingly, in still another 35 preferred aspect, the invention includes morphogens

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comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and accommodates the identities between the various
5 identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human
10 OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

II. Formulations and Methods for Administering Therapeutic Agents

15 The morphogens may be provided to an individual by any suitable means, preferably directly (e.g., locally, as by injection to a nerve tissue locus) or systemically (e.g., parenterally or orally). Where the
20 morphogen is to be provided parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or
25 by aerosol administration, the morphogen preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the
30 patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (9.85% NaCl, 0.15M), pH 7-7.4. The aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol
35 containing acetonitrile in 0.1% trifluoroacetic acid

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(TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble by association with a suitable molecule. For example, association of the mature dimer with the pro domain of the morphogen increases solubility of the protein significantly (see Section II.1, below). In fact, the endogenous protein is thought to be transported in this form. Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity. Biocompatible, preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen, polybutyrate, tricalcium phosphate, lactide and lactide/glycolide copolymers, may be useful excipients to control the release of the morphogen in vivo. Other potentially useful parenteral delivery systems for these morphogens include ethylene-vinyl acetate copolymer particles,

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osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example,

5 polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal

10 administration, methoxysalicylate for rectal administration, or cutric acid for vaginal administration.

Alternatively, the morphogens described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the

15 morphogens described herein typically are acid stable and protease-resistant (see, for example, U.S. Pat.No. 4,968,590.) In addition, at least one morphogen, OP-1, has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP-1 purified from

20 mammary gland extract is morphogenically active. Specifically, this protein induces endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard in vivo bone assay, such as is disclosed in

25

30 U.S. Pat.No. 4,968,590. Moreover, the morphogen also is detected in the bloodstream (see Example 9, below). Finally, soluble form morphogen, e.g., mature morphogen associated with the pro domain, is capable of maintaining neural pathways in a mammal (See Examples 4

35 and 6 below). These findings indicate that oral and

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parenteral administration are viable means for administering morphogens to an individual. In addition, while the mature forms of certain morphogens described herein typically are sparingly soluble, the 5 morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, morphogenically active form with part or all of the pro domain of the intact sequence and/or by association with one or more milk 10 components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

The compounds provided herein also may be 15 associated with molecules capable of targeting the morphogen or morphogen-stimulating agent to nerve tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on nerve tissue cells, 20 including neuronal or glial cells, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

25 As described above, the morphogens provided herein share significant sequence homology in the C-terminal active domains. By contrast, the sequences typically diverge significantly in the sequences which define the pro domain. Accordingly, the pro domain is thought to 30 be morphogen-specific. As described above, it is also known that the various morphogens identified to date are differentially expressed in the different tissues. Accordingly, without being limited to any given theory, it is likely that, under natural conditions in the 35 body, selected morphogens typically act on a given

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tissue. Accordingly, part or all of the pro domains which have been identified associated with the active form of the morphogen in solution, may serve as targeting molecules for the morphogens described

5 herein. For example, the pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Accordingly, another useful targeting molecule for targeting morphogen to nerve
10 tissue is part or all of a morphogen pro domain, particularly part or all of the pro domains of OP-1 or GDF-1, both of which proteins are found naturally associated with nerve tissue.

15 Finally, the morphogens or morphogen-stimulating agents provided herein may be administered alone or in combination with other molecules known to be beneficial in maintaining neural pathways, including nerve growth factors and anti-inflammatory agents.

20 The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and carriers. As noted above, such compositions may be
25 prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of powders, nasal drops, or aerosols.

30 The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations for a time
35 sufficient to eliminate or reduce the patient's

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pathological condition, to provide therapy for the neurological diseases and disorders described above, and amounts effective to enhance neural cell survival and/or to protect neurons and neural pathways in
5 anticipation of injury to nerve tissue.

As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a
10 number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug to be administered also is likely to depend on such
15 variables as the type and extent of progression of the neurological disease, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound excipients, and its route of administration. In general
20 terms, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a
25 preferred dose range is from about 0.1 μ g/kg to 100 mg/kg of body weight per day. Optimally, the morphogen dosage given in all cases is between 2-20 μ g of protein per kilogram weight of the patient per day. No obvious OP-1 induced pathological lesions are
30 induced when mature morphogen (e.g., OP-1, 20 μ g) is administered daily to normal growing rats for

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21 consecutive days. Moreover, 10 µg systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

5

Since the ability of proteins and protein fragments to penetrate the blood-brain barrier may be related to their size, lipophilicity or their net ionic charge, suitable modifications of the morphogens may be

10 formulated (e.g., by substituting pentafluorophenylalanine for phenylalanine, or by conjugation to a cationized protein such as albumin) to increase their transportability across the barrier, using standard methodologies known in the art. See,
15 for example, Kastin et al., Pharmac. Biochem. Behav. (1979) 11:713-716; Rapoport et al., Science (1980) 207:84-86; Pardridge et al., (1987) Biochem. Biophys. Res. Commun. 146:307-313; Riekkinen et al.,(1987) Peptides 8:261-265. The efficacy of these functional
20 analogs may be assessed for example, by evaluating the ability of these compounds to induce redifferentiation of transformed cells, or enhance survival of neurons at risk of dying, as described in the Examples provided herein.

25

In administering morphogens systemically in the methods of the present invention, preferably a large volume loading dose is used at the start of the treatment. The treatment then is continued with a
30 maintenance dose. Further administration then can be determined by monitoring at intervals the levels of the morphogen in the blood.

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Where injury to neurons of a neural pathway is induced deliberately as part of, for example, a surgical procedure, the morphogen preferably is provided just prior to, or concomitant with induction 5 of the trauma. Preferably, the morphogen is administered prophylactically in a surgical setting. Optimally, the morphogen dosage given in all cases is between 2-20 µg of protein per kilogram weight of the patient.

10

Alternatively, an effective amount of an agent capable of stimulating endogenous morphogen levels may be administered by any of the routes described above. For example, an agent capable of stimulating morphogen 15 production and/or secretion from nerve tissue cells may be provided to a mammal, e.g., by direct administration of the morphogen to glial cells associated with the nerve tissue to be treated. A method for identifying and testing agents capable of modulating the levels of 20 endogenous morphogens in a given tissue is described generally herein in Example 13, and in detail in international application US92/07359 (WO93/015172), the disclosure of which is incorporated herein by reference. Briefly, candidate compounds can be 25 identified and tested by incubating the compound in vitro with a test tissue or cells thereof, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the cells of that tissue. Here, 30 suitable tissue or cultured cells of a tissue preferably would comprise neurons and/or glial cells. For example, suitable tissue for testing may include cultured cells isolated from the substantia nigra, adendema glial cells, and the like.

35

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A currently preferred detection means for evaluating the level of the morphogen in culture upon exposure to the candidate compound comprises an immunoassay utilizing an antibody or other suitable 5 binding protein capable of reacting specifically with a morphogen and being detected as part of a complex with the morphogen. Immunoassays may be performed using standard techniques known in the art and antibodies raised against a morphogen and specific for that 10 morphogen. As described herein, morphogens may be isolated from natural-sourced material or they may be recombinantly produced. Agents capable of stimulating endogenous morphogens then may be formulated into pharmaceutical preparations and administered as 15 described herein.

Where the morphogen is to be provided to a site to stimulate axon regeneration, the morphogen preferably is provided to the site in association with a 20 biocompatible, preferably bioresorbable carrier suitable for maintaining a protein at a site in vivo, and through which a neural process may regenerate. A currently preferred carrier also comprises sufficient structure to assist direction of axonal growth. 25 Currently preferred carriers include structural molecules such as collagen, hyaluronic acid or laminin, and/or synthetic polymers or copolymers of, for example, polylactic acid, polyglycolic acid or polybutyric acid. Currently most preferred are 30 carriers comprising tissue extracellular matrix. These may be obtained commercially. In addition, a brain

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tissue-derived extracellular matrix may be prepared as described in international application US92/01968 (WO92/15323), incorporated hereinabove by reference, and/or by other means known in the art.

5

The currently preferred means for repairing breaks in neural pathways, particularly pathways of the peripheral nervous system, include providing the morphogen to the site as part of a device that includes
10 a biocompatible membrane or casing of a dimension sufficient to span the break and having openings adapted to receive severed nerve ends. The morphogen is disposed within the casing, preferably dispersed throughout a suitable carrier, and is accessible to the
15 severed nerve ends. Alternatively, the morphogen may be adsorbed onto the interior surface of the casing, or otherwise associated therewith. In addition, currently preferred casings have an impermeable exterior surface. The casing acts as a nerve guidance channel, aiding in
20 directing axonal growth. In addition, the casing also protects the damaged nerve from immunologically-related agents which may assist in scar tissue formation.
Suitable channel or casing materials include silicone or bioresorbable materials such as collagen, hyaluronic
25 acid, laminin, polylactic acid, polyglycolic acid, polybutyric acid and the like. Additionally, although the nerve guidance channels described herein generally are tubular in shape, it should be evident to those skilled in the art that various alternative shapes may
30 be employed. The lumen of the guidance channels may, for example, be oval or even square in cross section.

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Moreover the guidance channels may be constructed of two or more parts which may be clamped together to secure the nerve stumps. Nerve endings may be secured to the nerve guidance channels by means of sutures,

- 5 biocompatible adhesives such as fibrin glue, or other means known in the medical art.

II.1 Soluble Morphogen Complexes

- 10 A currently preferred form of the morphogen useful in therapeutic formulations, having improved solubility in aqueous solutions and consisting essentially of amino acids, is a dimeric morphogenic protein comprising at least the 100 amino acid peptide sequence
- 15 having the pattern of seven or more cysteine residues characteristic of the morphogen family complexed with a peptide comprising part or all of a pro region of a member of the morphogen family, or an allelic, species or other sequence variant thereof. Preferably, the
- 20 dimeric morphogenic protein is complexed with two peptides. Also, the dimeric morphogenic protein preferably is noncovalently complexed with the pro region peptide or peptides. The pro region peptides also preferably comprise at least the N-terminal
- 25 eighteen amino acids that define a given morphogen pro region. In a most preferred embodiment, peptides defining substantially the full length pro region are used.
- 30 Other soluble forms of morphogens include dimers of the uncleaved pro forms of these proteins, as well as "hemi-dimers" wherein one subunit of the dimer is an uncleaved pro form of the protein, and the other

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subunit comprises the mature form of the protein, including truncated forms thereof, preferably noncovalently associated with a cleaved pro domain peptide.

5

As described above, useful pro domains include the full length pro regions, as well as various truncated forms hereof, particularly truncated forms cleaved at proteolytic Arg-Xaa-Xaa-Arg cleavage sites. For example, in OP-1, possible pro sequences include sequences defined by residues 30-292 (full length form); 48-292; and 158-292. Soluble OP-1 complex stability is enhanced when the pro region comprises the full length form rather than a truncated form, such as the 48-292 truncated form, in that residues 30-47 show sequence homology to the N-terminal portions of other morphogens, and are believed to have particular utility in enhancing complex stability for all morphogens. Accordingly, currently preferred pro sequences are those encoding the full length form of the pro region for a given morphogen. Other pro sequences contemplated to have utility include biosynthetic pro sequences, particularly those that incorporate a sequence derived from the N-terminal portion of one or more morphogen pro sequences.

As will be appreciated by those having ordinary skill in the art, useful sequences encoding the pro region may be obtained from genetic sequences encoding known morphogens. Alternatively, chimeric pro regions can be constructed from the sequences of one or more known morphogens. Still another option is to create a synthetic sequence variant of one or more known pro region sequences.

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In another preferred aspect, useful pro region peptides include polypeptide chains comprising an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with a DNA or RNA sequence encoding at least the N-terminal eighteen amino acids of the pro region sequence for OP1 or OP2, e.g., nucleotides 136-192 and 152-211 of Seq. ID No. 16 and 20, respectively.

10 A. Isolation of Soluble morphogen complex from conditioned media or body fluid

Morphogens are expressed from mammalian cells as soluble complexes. Typically, however the complex is disassociated during purification, generally by exposure to denaturants often added to the purification solutions, such as detergents, alcohols, organic solvents, chaotropic agents and compounds added to reduce the pH of the solution. Provided below is a currently preferred protocol for purifying the soluble proteins from conditioned media (or, optionally, a body fluid such as serum, cerebro-spinal or peritoneal fluid), under non-denaturing conditions. The method is rapid, reproducible and yields isolated soluble morphogen complexes in substantially pure form.

Soluble morphogen complexes can be isolated from conditioned media using a simple, three step chromatographic protocol performed in the absence of denaturants. The protocol involves running the media (or body fluid) over an affinity column, followed by ion exchange and gel filtration chromatographies. The affinity column described below is a Zn-IMAC column. The present protocol has general applicability to the purification of a variety of morphogens, all of which

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are anticipated to be isolatable using only minor modifications of the protocol described below. An alternative protocol also envisioned to have utility an immunoaffinity column, created using standard

- 5 procedures and, for example, using antibody specific for a given morphogen pro domain (complexed, for example, to a protein A-conjugated Sepharose column.) Protocols for developing immunoaffinity columns are well described in the art, (see, for example, Guide to
10 Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly sections VII and XI.)

- In this experiment OP-1 was expressed in mammalian
15 CHO (chinese hamster ovary) cells as described in the art (see, for example, international application US90/05903 (WO91/05802).) The CHO cell conditioned media containing 0.5% FBS was initially purified using Immobilized Metal-Ion Affinity Chromatography (IMAC).
20 The soluble OP-1 complex from conditioned media binds very selectively to the Zn-IMAC resin and a high concentration of imidazole (50 mM imidazole, pH 8.0) is required for the effective elution of the bound complex. The Zn-IMAC step separates the soluble OP-1
25 from the bulk of the contaminating serum proteins that elute in the flow through and 35 mM imidazole wash fractions. The Zn-IMAC purified soluble OP-1 is next applied to an S-Sepharose cation-exchange column equilibrated in 20 mM NaPO₄ (pH 7.0) with 50 mM NaCl.
30 This S-Sepharose step serves to further purify and concentrate the soluble OP-1 complex in preparation for the following gel filtration step. The protein was

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applied to a Sephadryl S-200HR column equilibrated in TBS. Using substantially the same protocol, soluble morphogens also may be isolated from one or more body fluids, including serum, cerebro-spinal fluid or
5 peritoneal fluid.

IMAC was performed using Chelating-Sepharose (Pharmacia) that had been charged with three column volumes of 0.2 M ZnSO₄. The conditioned media was
10 titrated to pH 7.0 and applied directly to the Zn-IMAC resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM NaCl. The Zn-IMAC resin was loaded with 80 mL of starting conditioned media per mL of resin. After loading, the column was washed with equilibration
15 buffer and most of the contaminating proteins were eluted with 35 mM imidazole (pH 7.0) in equilibration buffer. The soluble OP-1 complex then is eluted with 50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM NaCl.
20

The 50 mM imidazole eluate containing the soluble OP-1 complex was diluted with nine volumes of 20 mM NaPO₄ (pH 7.0) and applied to an S-Sepharose (Pharmacia) column equilibrated in 20 mM NaPO₄ (pH 7.0)
25 with 50 mM NaCl. The S-Sepharose resin was loaded with an equivalent of 800 mL of starting conditioned media per mL of resin. After loading the S-Sepharose column was washed with equilibration buffer and eluted with 100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM
30 NaPO₄ (pH 7.0). The 300 mM NaCl pool was further purified using gel filtration chromatography. Fifty mls of the 300 mM NaCl eluate was applied to a 5.0 X 90 cm Sephadryl S-200HR (Pharmacia) equilibrated in Tris buffered saline (TBS), 50 mM Tris, 150 mM NaCl
35 (pH 7.4). The column was eluted at a flow rate of 5

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mL/minute collecting 10 mL fractions. The apparent molecular weight of the soluble OP-1 was determined by comparison to protein molecular weight standards (alcohol dehydrogenase (ADH, 150 kDa), bovine serum albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa) and cytochrome C (cyt C, 12.5 kDa). The purity of the S-200 column fractions was determined by separation on standard 15% polyacrylamide SDS gels stained with coomassie blue. The identity of the mature OP-1 and the pro-domain was determined by N-terminal sequence analysis after separation of the mature OP-1 from the pro-domain using standard reverse phase C18 HPLC.

The soluble OP-1 complex elutes with an apparent molecular weight of 110 kDa. This agrees well with the predicted composition of the soluble OP-1 complex with one mature OP-1 dimer (35-36 kDa) associated with two pro-domains (39 kDa each). Purity of the final complex can be verified by running the appropriate fraction in a reduced 15% polyacrylamide gel.

The complex components can be verified by running the complex-containing fraction from the S-200 or S-200HR columns over a reverse phase C18 HPLC column and eluting in an acetonitrile gradient (in 0.1% TFA), using standard procedures. The complex is dissociated by this step, and the pro domain and mature species elute as separate species. These separate species then can be subjected to N-terminal sequencing using standard procedures (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly pp. 602-613), and the identity of the isolated 36kD, 39kDa proteins confirmed as mature morphogen and isolated, cleaved pro domain, respectively. N-terminal sequencing of the

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isolated pro domain from mammalian cell produced OP-1 revealed 2 forms of the pro region, the intact form (beginning at residue 30 of Seq. ID No. 16) and a truncated form, (beginning at residue 48 of Seq. ID No. 16.) N-terminal sequencing of the polypeptide subunit of the isolated mature species reveals a range of N-termini for the mature sequence, beginning at residues 293, 300, 313, 315, 316, and 318, of Seq. ID No. 16, all of which are active as demonstrated by the standard bone induction assay.

B. In Vitro Soluble Morphogen Complex Formation

As an alternative to purifying soluble complexes from culture media or a body fluid, soluble complexes may be formulated from purified pro domains and mature dimeric species. Successful complex formation apparently requires association of the components under denaturing conditions sufficient to relax the folded structure of these molecules, without affecting disulfide bonds. Preferably, the denaturing conditions mimic the environment of an intracellular vesicle sufficiently such that the cleaved pro domain has an opportunity to associate with the mature dimeric species under relaxed folding conditions. The concentration of denaturant in the solution then is decreased in a controlled, preferably step-wise manner, so as to allow proper refolding of the dimer and pro regions while maintaining the association of the pro domain with the dimer. Useful denaturants include 4-6M urea or guanidine hydrochloride (GuHCl), in buffered solutions of pH 4-10, preferably pH 6-8. The soluble complex then is formed by controlled dialysis or dilution into a solution having a final denaturant concentration of less than 0.1-2M urea or GuHCl,

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preferably 1-2 M urea or GuHCl, which then preferably can be diluted into a physiological buffer. Protein purification/renaturing procedures and considerations are well described in the art, and details for

- 5 developing a suitable renaturing protocol readily can be determined by one having ordinary skill in the art. One useful text on the subject is Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly section V. Complex formation
10 also may be aided by addition of one or more chaperone proteins.

C. Stability of Soluble Morphogen Complexes

- 15 The stability of the highly purified soluble morphogen complex in a physiological buffer, e.g., tris-buffered saline (TBS) and phosphate-buffered saline (PBS), can be enhanced by any of a number of means. Currently preferred is by means of a pro region
20 that comprises at least the first 18 amino acids of the pro sequence (e.g., residues 30-47 of Seq. ID NO. 16 for OP-1), and preferably is the full length pro region. Residues 30-47 show sequence homology to the N-terminal portion of other morphogens and are believed
25 to have particular utility in enhancing complex stability for all morphogens. Other useful means for enhancing the stability of soluble morphogen complexes include three classes of additives. These additives include basic amino acids (e.g., L-arginine, lysine and
30 betaine); nonionic detergents (e.g., Tween 80 or NonIdet P-120); and carrier proteins (e.g., serum

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albumin and casein). Useful concentrations of these additives include 1-100 mM, preferably 10-70 mM, including 50 mM, basic amino acid; 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (v/v) nonionic 5 detergent; and 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (w/v) carrier protein.

III. Examples

10 Example 1. Identification of Morphogen-Expressing Tissue

Determining the tissue distribution of morphogens may be used to identify different morphogens expressed 15 in a given tissue, as well as to identify new, related morphogens. Tissue distribution also may be used to identify useful morphogen-producing tissue for use in screening and identifying candidate morphogen-stimulating agents. The morphogens (or their mRNA 20 transcripts) readily are identified in different tissues using standard methodologies and minor modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or 25 immunofluorescent techniques, and antibodies specific to the morphogen or morphogens of interest. Similarly, the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and transcript-specific probes.

30

Any probe capable of hybridizing specifically to a transcript, and distinguishing the transcript of interest from other, related transcripts may be used. Because the morphogens described herein share such high 35 sequence homology in their active, C-terminal domains,

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the tissue distribution of a specific morphogen transcript may best be determined using a probe specific for the pro region of the immature protein and/or the N-terminal region of the mature protein.

- 5 Another useful sequence is the 3' non-coding region flanking and immediately following the stop codon. These portions of the sequence vary substantially among the morphogens of this invention, and accordingly, are specific for each protein. For example, a particularly
- 10 useful Vgr-1-specific probe sequence is the PvuII-SacI fragment, a 265 bp fragment encoding both a portion of the untranslated pro region and the N-terminus of the mature sequence (see Lyons et al. (1989) PNAS 86:4554-4558 for a description of the cDNA sequence).
- 15 Similarly, particularly useful mOP-1-specific probe sequences are the BstX1-BglI fragment, a 0.68 Kb sequence that covers approximately two-thirds of the mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb sequence immediately upstream of the 7-cysteine domain;
- 20 and the Earl-PstI fragment, an 0.3 Kb fragment containing a portion of the 3'untranslated sequence (See Seq. ID No. 18, where the pro region is defined essentially by residues 30-291.) Similar approaches may be used, for example, with hOP-1 (Seq. ID No. 16)
- 25 or human or mouse OP-2 (Seq. ID Nos. 20 and 22.)

Using these morphogen-specific probes, which may be synthetically engineered or obtained from cloned sequences, morphogen transcripts can be identified in

- 30 mammalian tissue, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA is prepared from various adult murine tissues (e.g., liver, kidney, testis, heart, brain, thymus and stomach) by a standard methodology
- 35 such as by the method of Chomczyaski et al. ((1987)

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Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15 µg) 5 from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran membrane (Schleicher & Schuell). Following the transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 10 mW/cm²). Prior to hybridization, the appropriate probe is denatured by heating. The hybridization is carried out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours at 37°C using a hybridization mix of 40% 15 formamide, 5 x Denhardt's, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Examples demonstrating the tissue distribution of 20 various morphogens, including Vgr-1, OP-1, BMP2, BMP3, BMP4, BMP5, GDF-1, and OP-2 in developing and adult tissue are disclosed in international application US92/01968 (WO92/15323), and in Ozkaynak, et al., (1991) Biochem. Biophys. Res. Commn. 179:116-123, and 25 Ozkaynak, et al. (1992) J. Biol.Chem. 267: 25220-25227. Using the general probing methodology described herein, northern blot hybridizations using probes specific for these morphogens to probe brain, spleen, lung, heart, liver and kidney tissue indicate that kidney-related 30 tissue appears to be the primary expression source for OP-1, with brain, heart and lung tissues being secondary sources. Lung tissue appears to be the primary tissue expression source for Vgr-1, BMP5, BMP4 and BMP3. Lower levels of Vgr-1 also are seen in kidney 35 and heart tissue, while the liver appears to be a

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seen in kidney and heart tissue, while the liver appears to be a secondary expression source for BMP5, and the spleen appears to be a secondary expression source for BMP4. GDF-1 appears to be expressed 5 primarily in brain tissue. To date, OP-2 appears to be expressed primarily in early embryonic tissue. Specifically, northern blots of murine embryos and 6-day post-natal animals shows abundant OP2 expression in 8-day embryos. Expression is reduced significantly in 10 17-day embryos and is not detected in post-natal animals.

Example 2. Morphogen Localization in the Nervous System

15 Morphogens have been identified in developing and adult rat brain and spinal cord tissue, as determined both by northern blot hybridization of morphogen-specific probes to mRNA extracts from developing and 20 adult nerve tissue (see Example 1, above) and by immunolocalization studies. For example, northern blot analysis of developing rat tissue has identified significant OP-1 mRNA transcript expression in the CNS international application US92/01968 (WO92/15323), and 25 Ozkaynak et al. (1991) Biochem. Biophys. Res. Comm., 179:11623 and Ozkaynak, et al. (1992) J. Biol. Chem. 267:25220-25227. GDF-1 mRNA appears to be expressed primarily in developing and adult nerve tissue, specifically in the brain, including the cerebellum and 30 brain stem, spinal cord and peripheral nerves (Lee, S. (1991) PNAS 88: 4250-4254). BMP2B (also referred in the art as BMP4) and Vgr-1 transcripts also have been reported to be expressed in nerve tissue (Jones et al. (1991) Development 111:531-542), although the nerve 35 tissue does not appear to be the primary expression

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tissue for these genes (Ozkaynak, et al., (1992) J. Biol. Chem. 267:25220-25227. Specifically, CBMP2 transcripts are reported in the region of the diencephalon associated with pituitary development, and

- 5 Vgr-1 transcripts are reported in the anteroposterior axis of the CNS, including in the roof plate of the developing neural tube, as well as in the cells immediately adjacent the floor plate of the developing neural tube. In older rats, Vgr-1 transcripts are
10 reported in developing hippocampus tissue. In addition, the genes encoding OP-1 and BMP2 originally were identified by probing human hippocampus cDNA libraries.

- 15 Immunolocalization studies, performed using standard methodologies known in the art and disclosed in international application US92/01968 (WO92/15323), the disclosure of which is incorporated herein, localized OP-1 expression to particular areas of
20 developing and adult rat brain and spinal cord tissue. Specifically, OP-1 protein expression was assessed in adult (2-3 months old) and five or six-day old mouse embryonic nerve tissue, using standard morphogen-specific antisera, specifically, rabbit anti-OP1
25 antisera, made using standard antibody protocols known in the art and preferably purified on an OP-1 affinity column. The antibody itself was labelled using standard fluorescent labelling techniques, or a labelled anti-rabbit IgG molecule was used to visualize
30 bound OP-1 antibody.

As can be seen in FIG 1A and 1B, immunofluorescence staining demonstrates the presence of OP-1 in adult rat central nervous system (CNS.) Similar and extensive
35 staining is seen in both the brain (1A) and spinal cord

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- (1B). OP-1 appears to be localized predominantly to the extracellular matrix of the grey matter (neuronal cell bodies), distinctly present in all areas except the cell bodies themselves. In white matter, formed 5 mainly of myelinated nerve fibers, staining appears to be confined to astrocytes (glial cells). A similar staining pattern also was seen in newborn rat (10 day old) brain sections.
- 10 In addition, OP-1 has been specifically localized in the substantia nigra, which is composed primarily of striatal basal ganglia, a system of accessory motor neurons that function in association with the cerebral cortex and corticospinal system. Dysfunctions in this 15 subpopulation or system of neurons are associated with a number of neuropathies, including Huntington's chorea and Parkinson's disease.
- OP1 also has been localized at adendema glial 20 cells, known to secrete factors into the cerebrospinal fluid, and which occur around the intraventricular valve, coroid fissure, and central canal of the brain in both developing and adult rat.
- 25 Finally, morphogen inhibition in developing embryos inhibits nerve tissue development. Specifically, 9-day mouse embryo cells, cultured in vitro under standard culturing conditions, were incubated in the presence and absence of an OP-1-specific monoclonal antibody 30 prepared using recombinantly produced, purified mature OP-1 and the immunogen. The antibody was prepared using standard antibody production means well known in the art and as described generally in Example 13. After two days, the effect of the antibody on the 35 developing embryo was evaluated by histology. As

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- determined by histological examination, the OP-1-specific antibody specifically inhibits eye lobe formation in the developing embryo. In particular, the diencephalon outgrowth does not develop. In addition,
- 5 the heart is malformed and enlarged. Moreover, in separate immunolocalization studies on embryo sections with labelled OP-1 specific antibody, the OP-1-specific antibody localizes to neural epithelia.
- 10 The endogenous morphogens which act on neuronal cells may be expressed and secreted by nerve tissue cells, e.g., by neurons and/or glial cells associated with the neurons, and/or they may be transported to the neurons by the cerebrospinal fluid and/or bloodstream.
- 15 Recently, OP-1 has been identified in the human blood (See Example 9, below). In addition, transplanted Schwann cells recently have been shown to stimulate nerve fiber formation in rat spinal cord, including inducing vascularization and myelin sheath formation
- 20 around at least some of the new neuronal processes (Bunge (1991) Exp. Neurology 114:254-257.) The regenerative property of these cells may be mediated by the secretion of a morphogen by the Schwann cells.
- 25 Example 3. Morphogen Enhancement of Neuronal Cell Survival
- The morphogens described herein enhance cell survival, particularly of neuronal cells at risk of
- 30 dying. For example, fully differentiated neurons are non-mitotic and die in vitro when cultured under standard mammalian cell culture conditions, using a chemically defined or low serum medium known in the art, (see, for example, Charness (1986) J. Biol. Chem.
- 35 26:3164-3169 and Freese et al. (1990) Brain Res.

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521:254-264.) However, if a primary culture of non-mitotic neuronal cells is treated with a morphogen, the survival of these cells is enhanced significantly. For example, a primary culture of striatal basal ganglia isolated from the substantia nigra of adult rat brain was prepared using standard procedures, e.g., by dissociation by trituration with pasteur pipette of substantia nigra tissue, using standard tissue culturing protocols, and grown in a low serum medium, e.g., containing 50% DMEM (Dulbecco's modified Eagle's medium), 50% F-12 medium, heat inactivated horse serum supplemented with penicillin/streptomycin and 4 g/l glucose. Under standard culture conditions, these cells are undergoing significant cell death by three weeks when cultured in a serum-free medium. Cell death is evidenced morphologically by the inability of cells to remain adherent and by changes in their ultrastructural characteristics, e.g., by chromatin clumping and organelle disintegration.

20

In this example, the cultured basal ganglia were treated with chemically defined medium conditioned with 0.1-100 ng/ml OP-1. Fresh, morphogen-conditioned medium was provided to the cells every 3-4 days. Cell survival was enhanced significantly and was dose dependent upon the level of OP-1 added: cell death decreased significantly as the concentration of OP-1 was increased in cell cultures. Specifically, cells remained adherent and continued to maintain the morphology of viable differentiated neurons. In the absence of morphogen treatment, the majority of the cultured cells dissociated and underwent cell necrosis.

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Dysfunctions in the basal ganglia of the substantia nigra are associated with Huntington's chorea and parkinsonism in vivo. The ability of the morphogens defined herein to enhance neuron survival indicates

- 5 that these morphogens will be useful as part of a therapy to enhance survival of neuronal cells at risk of dying in vivo due, for example, to a neuropathy or chemical or mechanical trauma. It is particularly anticipated that these morphogens will provide a useful
- 10 therapeutic agent to treat neuropathies which affect the striatal basal ganglia, including Huntington's chorea and Parkinson's disease. For clinical applications, the morphogen may be administered or, alternatively, a morphogen-stimulating agent may be
- 15 administered.

Example 4. Morphogen-Induced Redifferentiation of Transformed Cells

20

The morphogens described herein also induce redifferentiation of transformed cells to a morphology characteristic of untransformed cells. In particular, the morphogens are capable of inducing

- 25 redifferentiation of transformed cells of neuronal origin to a morphology characteristic of untransformed neurons. The example provided below details morphogen induced redifferentiation of a transformed human cell line of neuronal origin, NG105-115. Morphogen-induced
- 30 redifferentiation of transformed cells also has been shown in mouse neuroblastoma cells (N1E-115) and in human embryo carcinoma cells (see international application US92/01968 (WO92/15323)).

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NG108-15 is a transformed hybrid cell line produced by fusing neuroblastoma x glioma cells (obtained from America Type Tissue Culture, Rockville, MD), and exhibiting a morphology characteristic of transformed 5 embryonic neurons, e.g., having a fibroblastic morphology. Specifically, the cells have polygonal cell bodies, short, spike-like processes and make few contacts with neighboring cells (see FIG. 1A).
Incubation of NG108-15 cells, cultured in a chemically 10 defined, serum-free medium, with 0.1 to 300 ng/ml of OP-1 for four hours induces an orderly, dose-dependent change in cell morphology.

In the experiment NG108-15 cells were subcultured 15 on poly-L-lysine coated 6-well plates. Each well contained 40-50,000 cells in 2.5 ml of chemically defined medium. On the third day 2.5 μ l of OP-1 in 60% ethanol containing 0.025% trifluoroacetic acid was added to each well. OP-1 concentrations of 0-300 ng/ml were 20 tested. Typically, the media was changed daily with new aliquots of OP-1, although morphogenesis can be induced by a single four hour incubation with OP-1. In addition, OP-1 concentrations of 10 ng/ml were sufficient to induce redifferentiation. After one day, 25 hOP-1-treated cells undergo a significant change in their cellular ultrastructure, including rounding of the soma, increase in phase brightness and extension of the short neurite processes. After two days, cells treated with OP-1 begin to form epithelioid sheets, 30 which provide the basis for the growth of multilayered aggregates at three day's post-treatment. By four days, the great majority of OP-1-treated cells are associated in tightly-packed, multilayered aggregates

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(Fig. 1B). Fig. 2 plots the mean number of multi-layered aggregates or cell clumps identified in twenty randomly selected fields from six independent experiments, as a function of morphogen concentration.

- 5 Forty ng/ml of OP-1 is sufficient for half maximum induction of cell aggregation.

The morphogen-induced redifferentiation occurred without any associated changes in DNA synthesis, cell division, or cell viability, making it unlikely that the morphologic changes were secondary to cell differentiation or a toxic effect of hOP-1. Moreover, the OP-1-induced morphogenesis does not inhibit cell division, as determined by ³H-thymidine uptake, unlike other molecules which have been shown to stimulate differentiation of transformed cells, such as butyrate, DMSO, retanoic acid or Forskolin. The data indicate that OP-1 can maintain cell stability and viability after inducing redifferentiation. In addition, the effects are morphogen specific, and redifferentiation is not induced when NG108-15 cells are incubated with 0.1-40 ng/ml TGF- β .

The experiments also have been performed with highly purified soluble morphogen (e.g., mature OP1 associated with its pro domain) which also specifically induced redifferentiation of NG108-15 cells.

The morphogens described herein accordingly provide useful therapeutic agents for the treatment of neoplasias and neoplastic lesions of the nervous system, particularly in the treatment of

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neuroblastomas, including retinoblastomas, and gliomas. The morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

5

Example 5. Nerve Tissue Protection from Chemical Trauma

10 The ability of the morphogens described herein to enhance survival of neuronal cells and to induce cell aggregation and cell-cell adhesion in redifferentiated cells, indicates that the morphogens will be useful as therapeutic agents to maintain neural pathways by
15 protecting the cells defining the pathway from the damage caused by chemical trauma. In particular, the morphogens can protect neurons, including developing neurons, from the effects of toxins known to inhibit the proliferation and migration of neurons and to
20 interfere with cell-cell adhesion. Examples of such toxins include ethanol, one or more of the toxins present in cigarette smoke, and a variety of opiates. The toxic effects of ethanol on developing neurons induces the neurological damage manifested in fetal
25 alcohol syndrome. The morphogens also may protect neurons from the cytoxic effects associated with excitatory amino acids such as glutamate.

For example, ethanol inhibits the cell-cell
30 adhesion effects induced in morphogen-treated NG108-15 cells when provided to these cells at a concentration of 25-50 mM. Half maximal inhibition can be achieved with 5-10 mM ethanol, the concentration of blood alcohol in an adult following ingestion of a single
35 alcoholic beverage. Ethanol likely interferes with the

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homophilic binding of CAMs between cells, rather than their induction, as morphogen-induced N-CAM levels are unaffected by ethanol. Moreover, the inhibitory effect is inversely proportional to morphogen concentration.

- 5 Accordingly, it is envisioned that administration of a morphogen or morphogen-stimulating agent to neurons, particularly developing neurons, at risk of damage from exposure to toxins such as ethanol, may protect these cells from nerve tissue damage by overcoming the
10 toxin's inhibitory effects. The morphogens described herein also are useful in therapies to treat damaged neural pathways resulting from a neuropathy induced by exposure to these toxins.

15

Example 6. Morphogen-Induced CAM Expression

The morphogens described herein induce CAM expression, particularly N-CAM expression, as part of
20 their induction of morphogenesis. CAMs are morphoregulatory molecules identified in all tissues as an essential step in tissue development. N-CAMs, which comprise at least 3 isoforms (N-CAM-180, N-CAM-140 and N-CAM-120, where "180", "140" and "120" indicate the
25 apparent molecular weights of the isoforms as measured by polyacrylamide gel electrophoresis) are expressed at least transiently in developing tissues, and permanently in nerve tissue. Both the N-CAM-180 and N-CAM-140 isoforms are expressed in both developing and
30 adult tissue. The N-CAM-120 isoform is found only in adult tissue. Another neural CAM is L1.

N-CAMs are implicated in appropriate neural development, including appropriate nuerulation,
35 neuronal migration, fasciculation, and synaptogenesis.

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- Inhibition of N-CAM production, as by complexing the molecule with an N-CAM-specific antibody, inhibits retina organization, including retinal axon migration, and axon regeneration in the peripheral nervous system,
- 5 as well as axon synapsis with target muscle cells. In addition, significant evidence indicates that physical or chemical trauma to neurons, oncogenic transformation and some genetic neurological disorders are accompanied by changes in CAM expression, which alter the adhesive
- 10 or migratory behavior of these cells. Specifically, increased N-CAM levels are reported in Huntington's disease striatum (e.g., striatal basal ganglia), and decreased adhesion is noted in Alzheimer's disease.
- 15 The morphogens described herein can stimulate CAM production, particularly L1 and N-CAM production, including all three isoforms of the N-CAM molecule. For example, N-CAM expression is stimulated significantly in morphogen-treated NG108-15 cells.
- 20 Untreated NG108-15 cells exhibit a fibroblastic, or minimally differentiated morphology and express only the 180 and 140 isoforms of N-CAM normally associated with a developing cell. Following morphogen treatment these cells exhibit a morphology characteristic of
- 25 adult neurons and express enhanced levels of all three N-CAM isoforms. Using a similar protocol as described in the example below, morphogen treatment of NG108-15 cells also induced L1 expression.
- 30 In this example NG108-15 cells were cultured for 4 days in the presence of increasing concentrations of OP-1 and standard Western blots performed on whole cells extracts. N-CAM isoforms were detected with an antibody which crossreacts with all three isoforms,
- 35 mAb H28.123, obtained from Sigma Chemical Co.,

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St. Louis, the different isoforms being distinguishable by their different mobilities on an electrophoresis gel. Control NG108-15 cells (untreated) express both the 140 kDa and the 180 kDa isoforms, but not the 120
5 kDa, as determined by western blot analyses using up to 100 µg of protein. Treatment of NG108-15 cells with OP-1 resulted in a dose-dependent increase in the expression of the 180 kDa and 140 kDa isoforms, as well as the induction of the 120 kDa isoform. See Fig. 2A
10 and 2B. Fig. 2B is a Western blot of OP1-treated NG108-15 cell extracts, probed with mAb H28.123, showing the induction of all three isoforms. Fig. 2A is a dose response curve of N-CAM-180 and N-CAM-140 induction as a function of morphogen concentration. N-
15 CAM-120 is not shown in the graph as it could not be quantitated in control cells. However, as is clearly evident from the Western blot in Fig. 2A, N-CAM-120 is induced in response to morphogen treatment. The differential induction of N-CAM 180 and 140 isoforms
20 seen may be because constitutive expression of the 140 isoform is close to maximum.

The increase in N-CAM expression corresponded in a dose-dependent manner with the morphogen induction of
25 multicellular aggregates. Compare Fig. 2A and Fig 3. Fig. 3 graphs the mean number of multilayered aggregates (clumps) counted per 20 randomly selected fields in 6 independent experiments, versus the concentration of morphogen. The induction of the 120
30 isoform also indicates that morphogen-induced redifferentiation of transformed cells stimulates not only redifferentiation of these cells from a transformed phenotype, but also differentiation to a phenotype corresponding to a developed cell. Standard
35 immunolocalization studies performed with the mAb

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- H28.123 on morphogen-treated cells show N-CAM cluster formation associated with the periphery and processes of treated cells and no reactivity with untreated cells. Moreover, morphogen treatment does not appear
- 5 to inhibit cell division as determined by cell counting or ^3H -thymidine uptake. Finally, known chemical differentiating agents, such as Forskolin and dimethylsulfoxide do not induce N-CAM production.
- 10 In addition, the cell aggregation effects of OP-1 on NG108-15 cells can be inhibited with anti-N-CAM antibodies or antisense N-CAM oligonucleotides. Antisense oligonucleotides can be made synthetically on a nucleotide synthesizer, using standard means known in
- 15 the art. Preferably, phosphorothioate oligonucleotides ("S-oligos") are prepared, to enhance transport of the nucleotides across cell membranes. Concentrations of both N-CAM antibodies and N-CAM antisense oligonucleotides sufficient to inhibit N-CAM induction
- 20 also inhibited formation of multilayered cell aggregates. Specifically, incubation of morphogen-treated NG108-115 cells with 0.3-3 μM N-CAM antisense S-oligos, 5-500 μM unmodified N-CAM antisense oligos, or 10 $\mu\text{g}/\text{ml}$ mAb H28.123 significantly inhibits cell
- 25 aggregation. It is likely that morphogen treatment also stimulates other CAMs, as inhibition is not complete.

- The experiments also have been performed with
- 30 soluble morphogen (e.g., mature OP-1 associated with its pro domain) which also specifically induced CAM expression.

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The morphogens described herein are useful as therapeutic agents to treat neurological disorders associated with altered CAM levels, particularly N-CAM levels, such as Huntington's chorea and Alzheimers' disease, and the like. In clinical applications, the morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

10 The efficacy of the morphogens described herein to affect N-CAM expression may be assessed in vitro using a suitable cell line and the methods described herein. In addition to a transformed cell line, N-CAM expression can be assayed in a primary cell culture of
15 neural or glial cells, following the procedures described herein. The efficacy of morphogen treatment on N-CAM expression in vivo may be evaluated by tissue biopsy as described in Example 9, below, and detecting N-CAM molecules with an N-CAM-specific antibody, such
20 as mAb H28.123, or using the animal model described in Example 11.

Alternatively, the level of N-CAM proteins or protein fragments present in cerebrospinal fluid or
25 serum also may be detected to evaluate the effect of morphogen treatment. N-CAM molecules are known to slough off cell surfaces and have been detected in both serum and cerebrospinal fluid. In addition, altered levels of the soluble form of N-CAM are associated with
30 normal pressure hydrocephalus and type II schizophrenia. N-CAM fluid levels may be detected following the procedure described in Example 9 and using an N-CAM specific antibody, such as mAb H28.123.

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Example 7. Morphogen-Induced Nerve Gap Repair (PNS)

The morphogens described herein also stimulate peripheral nervous system axonal growth over extended distances allowing repair and regeneration of damaged neural pathways. While neurons of the peripheral nervous system can sprout new processes following injury, without guidance these sproutings typically fail to connect appropriately and die. Where the break is extensive, e.g., greater than 5 or 10 mm, regeneration is poor or nonexistent.

In this example morphogen stimulation of nerve regeneration was assessed using the rat sciatic nerve model. The rat sciatic nerve can regenerate spontaneously across a 5 mm gap, and occasionally across a 10 mm gap, provided that the severed ends are inserted in a saline-filled nerve guidance channel. In this experiment, nerve regeneration across a 12mm gap was tested.

Adult female Sprague-Dawley rats (Charles River Laboratories, Inc.) weighing 230-250 g were anesthetized with intraperitoneal injections of sodium pentobarbital 35 mg/kg body weight). A skin incision was made parallel and just posterior to the femur. The avascular intermuscular plane between vastus lateralis and hamstring muscles were entered and followed to the loose fibroareolar tissue surrounding the sciatic nerve. The loose tissue was divided longitudinally thereby freeing the sciatic nerve over its full extent without devascularizing any portion. Under a surgical

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microscope the sciatic nerves were transected with microscissors at mid-thigh and grafted with an OP-1 gel graft that separated the nerve stumps by 12 mm. The graft region was encased in a silicone tube 20 mm in 5 length with a 1.5 mm inner diameter, the interior of which was filled a morphogen solution. Specifically, The central 12 mm of the tube consisted of an OP-1 gel prepared by mixing 1 to 5 µg of substantially pure CHO-produced recombinant OP-1 with approximately 100 µl of 10 MATRIGEL™ (from Collaborative Research, Inc., Bedford, MA), an extracellular matrix extract derived from mouse sarcoma tissue, and containing solubilized tissue basement membrane, including laminin, type IV collagen, heparin sulfate, proteoglycan and entactin, in 15 phosphate-buffered saline. The OP-1-filled tube was implanted directly into the defect site, allowing 4 mm on each end to insert the nerve stumps. Each stump was abutted against the OP-1 gel and was secured in the silicone tube by three stitches of commercially 20 available surgical 10-0 nylon through the epineurium, the fascicle protective sheath.

In addition to OP-1 gel grafts, empty silicone tubes, silicone tubes filled with gel only and 25 "reverse" autografts, wherein 12 mm transected segments of the animal's sciatic nerve were rotated 180° prior to suturing, were grafted as controls. All experiments were performed in quadruplicate. All wounds were closed by wound clips that were removed after 10 days. 30 All rats were grafted on both legs. At 3 weeks the animals were sacrificed, and the grafted segments removed and frozen on dry ice immediately. Frozen

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sections then were cut throughout the graft site, and examined for axonal regeneration by immunofluorescent staining using anti-neurofilament antibodies labeled with flurocein (obtained from Sigma Chemical Co.,

5 St. Louis).

Regeneration of the sciatic nerve occurred across the entire 12 mm distance in all graft sites wherein the gap was filled with the OP-1 gel. By contrast, 10 empty silicone tubes and reverse autografts did not show nerve regeneration, and only one graft site containing the gel alone showed axon regeneration.

15 Example 8. Morphogen-Induced Nerve Gap Repair (CNS)

Following axonal damage in vivo the CNS neurons are unable to resprout processes. Accordingly, trauma to CNS nerve tissue, including the spinal cord, optic 20 nerve and retina, severely damages or destroys the neural pathways defined by these cells. Peripheral nerve grafts have been used in an effort to bypass CNS axonal damage. Successful autologous graft repair to date apparently requires that the graft site occur near 25 the CNS neuronal cell body, and a primary result of CNS axotomy is neuronal cell death. The efficacy of morphogens described herein on CNS nerve repair, may be evaluated using a rat crushed optic nerve model such as the one described by Bignami et al., (1979) Exp. Eye 30 Res. 28: 63-69, the disclosure of which is incorporated herein by reference. Briefly, and as described therein, laboratory rats (e.g., from Charles River Laboratories, Wilmington, MA) are anesthetized using standard surgical procedures, and the optic nerve 35 crushed by pulling the eye gently out of the orbit,

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inserting a watchmaker forceps behind the eyeball and squeezing the optic nerve with the forceps for 15 seconds, followed by a 30 second interval and second 15 second squeeze. Rats are sacrificed at different 5 time intervals, e.g., at 48 hours, and at 3, 4, 11, 15 and 18 days after operation. The effect of morphogen on optic nerve repair can be assessed by performing the experiment in duplicate and providing morphogen or PBS (e.g., 25 μ l solution, and 25 μ g morphogen) to the 10 optic nerve, e.g., just prior to the operation, concomitant with the operation, or at specific times after the operation.

In the absence of therapy, the surgery induces 15 glial scarring of the crushed nerve, as determined by immunofluorescence staining for glial fibrillary acidic protein (GFA), a marker protein for glial scarring, and by histology. Indirect immunofluorescence on air-dried cryostat sections as described in Bignami et al. (1974) 20 J. Comp. Neur. 153: 27-38, using commercially available antibodies to GFA (e.g., Sigma Chemical Co., St. Louis). Reduced levels of GFA are anticipated in animals treated with the morphogen, evidencing the ability of morphogens to inhibit glial scar formation 25 and to stimulate optic nerve regeneration.

Example 9. Nerve Tissue Diagnostics

Morphogen localization in nerve tissue can be used 30 as part of a method for diagnosing a neurological disorder or neuropathy. The method may be particularly advantageous for diagnosing neuropathies of brain tissue. Specifically, a biopsy of brain tissue is performed on a patient at risk, using standard 35 procedures known in the medical art. Morphogen

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expression associated with the biopsied tissue then is assessed using standard methodologies, as by immunolocalization, using standard immunofluorescence techniques in concert with morphogen-specific antisera or monoclonal antibodies. Specifically, the biopsied tissue is thin sectioned using standard methodologies known in the art, and fluorescently labelled (or otherwise detectable) antibodies incubated with the tissue under conditions sufficient to allow specific antigen-antibody complex formation. The presence and quantity of complex formed then is detected and compared with a predetermined standard or reference value. Detection of altered levels of morphogen present in the tissue then may be used as an indicator of tissue dysfunction. Alternatively, fluctuation in morphogen levels may be assessed by monitoring morphogen transcription levels, either by standard northern blot analysis or in situ hybridization, using a labelled probe capable of hybridizing specifically to morphogen RNA and standard RNA hybridization protocols well described in the art.

Fluctuations in morphogen levels present in the cerebrospinal fluid or bloodstream also may be used to evaluate nerve tissue viability. For example, morphogens are detected associated with adenoma cells which are known to secrete factors into the cerebrospinal fluid, and are localized generally associated with glial cells, and in the extracellular matrix, but not with neuronal cell bodies.

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Accordingly, the cerebrospinal fluid may be a natural means of morphogen transport. Alternatively, morphogens may be released from dying cells into cerebrospinal fluid. In addition, OP-1 recently has 5 been identified in human blood, which also may be a means of morphogen transport, and/or a repository for the contents of dying cells.

- Spinal fluid may be obtained from an individual by 10 a standard lumbar puncture, using standard methodologies known in the medical art. Similarly, serum samples may be obtained by standard venipuncture and serum prepared by centrifugation at 3,000 RPM for ten minutes. The presence of morphogen in the serum or 15 cerebral spinal fluid then may be assessed by standard Western blot (immunoblot), ELISA or RIA procedures. Briefly, for example, with the ELISA, samples may be diluted in an appropriate buffer, such as phosphate-buffered saline, and 50 µl aliquots allowed to absorb 20 to flat bottomed wells in microtitre plates pre-coated with morphogen-specific antibody, and allowed to incubate for 18 hours at 4°C. Plates then may be washed with a standard buffer and incubated with 50 µl aliquots of a second morphogen-specific antibody 25 conjugated with a detecting agent, e.g., biotin, in an appropriate buffer, for 90 minutes at room temperature. Morphogen-antibody complexes then may be detected using standard procedures.
- 30 Alternatively, a morphogen-specific affinity column may be created using, for example, morphogen-specific antibodies adsorbed to a column matrix, and passing the fluid sample through the matrix to selectively extract the morphogen of interest. The morphogen then is 35 eluted. A suitable elution buffer may be determined

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empirically by determining appropriate binding and elution conditions first with a control (e.g., purified, recombinantly-produced morphogen.) Fractions then are tested for the presence of the morphogen by 5 standard immunoblot, and confirmed by N-terminal sequencing. Morphogen concentrations in serum or other fluid samples then may be determined using standard protein quantification techniques, including by spectrophotometric absorbance or by quantitation by 10 ELISA or RIA antibody assays. Using this procedure, OP-1 has been identified in serum.

OP-1 was detected in human serum using the following assay. A monoclonal antibody raised against 15 mammalian, recombinantly produced OP-1 using standard immunology techniques well described in the art and described generally in Example 13, was immobilized by passing the antibody over an activated agarose gel (e.g., Affi-Gel™, from Bio-Rad Laboratories, Richmond, 20 CA, prepared following manufacturer's instructions), and used to purify OP-1 from serum. Human serum then was passed over the column and eluted with 3M K-thiocyanate. K-thiocyanante fractions then were dialyzed in 6M urea, 20mM PO₄, pH 7.0, applied to a C8 25 HPLC column, and eluted with a 20 minute, 25-50% acetonitrile/0.1% TFA gradient. Mature, recombinantly produced OP-1 homodimers elute between 20-22 minutes. Fractions then were collected and tested for the 30 presence of OP-1 by standard immunoblot. Fig. 4 is an immunoblot showing OP-1 in human sera under reducing and oxidized conditions. In the figure, lanes 1 and 4 are OP-1 standards, run under oxidized (lane 1) and

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reduced (lane 4) conditions. Lane 5 shows molecular weight markers at 17, 27 and 39 kDa. Lanes 2 and 3 are human sera OP-1, run under oxidized (lane 2) and reduced (lane 3) conditions.

5

Morphogens may be used in diagnostic applications by comparing the quantity of morphogen present in a body fluid sample with a predetermined reference value, with fluctuations in fluid morphogen levels indicating 10 a change in the status of nerve tissue. Alternatively, fluctuations in the level of endogenous morphogen antibodies may be detected by this method, most likely in serum, using an antibody or other binding protein capable of interacting specifically with the endogenous 15 morphogen antibody. Detected fluctuations in the levels of the endogenous antibody may be used as indicators of a change in tissue status.

20 Example 10. Alleviation of Immune Response-Mediated Nerve Tissue Damage

The morphogens described herein may be used to alleviate immunologically-related damage to nerve 25 tissue. Details of this damage and the use of morphogens to alleviate this injury are disclosed in international application US92/07358 (WO93/04692). A primary source of such damage to nerve tissue follows hypoxia or ischemia-reperfusion of a blood supply to a 30 neural pathway, such as may result from an embolic stroke, or may be induced during a surgical procedure.

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- As described in international application US92/07358 (WO93/04692), morphogens have been shown to alleviate damage to myocardial tissue following ischemia-reperfusion of the blood supply to the tissue. The 5 effect of morphogens on alleviating immunologically-related damage to nerve tissue may be assessed using methodologies and models known to those skilled in the art and described below.
- 10 For example, the rabbit embolic stroke model provides a useful method for assessing the effect of morphogens on tissue injury following cerebral ischemia-reperfusion. The protocol disclosed below is essentially that of Phillips et al. (1989) Annals of 15 Neurology 25:281-285, the disclosure of which is herein incorporated by reference. Briefly, white New England rabbits (2-3kg) are anesthetized and placed on a respirator. The intracranial circulation then is selectively catheterized by the Seldinger technique.
- 20 Baseline cerebral angiography then is performed, employing a digital substration unit. The distal internal carotid artery or its branches then is selectively embolized with 0.035 ml of 18-hour-aged autologous thrombus. Arterial occlusion is documented 25 by repeat angiography immediately after embolization. After a time sufficient to induce cerebral infarcts (15 minutes or 90 minutes), reperfusion is induced by administering a bolus of a reperfusion agent such as the TPA analogue FB-FB-CF (e.g., 0.8 mg/kg over 2 30 minutes).

The effect of morphogen on cerebral infarcts can be assessed by administering varying concentrations of morphogens, e.g., OP-1, at different times following 35 embolization and/or reperfusion. The rabbits are

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sacrificed 3-14 days post embolization and their brains prepared for neuropathological examination by fixing by immersion in 10% neutral buffered formalin for at least 2 weeks. The brains then are sectioned in a coronal plane at 2-3 mm intervals, numbered and submitted for standard histological processing in paraffin, and the degree of nerve tissue necrosis determined visually. Morphogen-treated animals are anticipated to reduce or significantly inhibit nerve tissue necrosis following cerebral ischemia-reperfusion in the test animals as determined by histology comparison with nontreated animals.

15 Example 11. Animal Model for Assessing Morphogen Efficacy In Vivo

The in vivo activities of the morphogens described herein also are assessed readily in an animal model as described herein. A suitable animal, preferably exhibiting nerve tissue damage, for example, genetically or environmentally induced, is injected intracerebrally with an effective amount of a morphogen in a suitable therapeutic formulation, such as phosphate-buffered saline, pH 7. The morphogen preferably is injected within the area of the affected neurons. The affected tissue is excised at a subsequent time point and the tissue evaluated morphologically and/or by evaluation of an appropriate biochemical marker (e.g., by morphogen or N-CAM localization; or by measuring the dose-dependent effect on a biochemical marker for CNS neurotrophic activity or for CNS tissue damage, using for example, glial fibrillary acidic protein as the marker. The dosage

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and incubation time will vary with the animal to be tested. Suitable dosage ranges for different species may be determined by comparison with established animal models. Presented below is an exemplary protocol for
5 a rat brain stab model.

Briefly, male Long Evans rats, obtained from standard commercial sources, are anesthetized and the head area prepared for surgery. The calvariae is
10 exposed using standard surgical procedures and a hole drilled toward the center of each lobe using a 0.035K wire, just piercing the calvariae. 25 μ l solutions containing either morphogen (e.g., OP-1, 25 μ g) or PBS then is provided to each of the holes by Hamilton
15 syringe. Solutions are delivered to a depth approximately 3 mm below the surface, into the underlying cortex, corpus callosum and hippocampus. The skin then is sutured and the animal allowed to recover.
20

Three days post surgery, rats are sacrificed by decapitation and their brains processed for sectioning. Scar tissue formation is evaluated by immunofluorescence staining for glial fibrillary acidic protein, a marker
25 protein for glial scarring, to qualitatively determine the degree of scar formation. Glial fibrillary acidic protein antibodies are available commercially, e.g., from Sigma Chemical Co., St. Louis, MO. Sections also are probed with anti-OP-1 antibodies to determine the
30 presence of OP-1. Reduced levels of glial fibrillary acidic protein are anticipated in the tissue sections of animals treated with the morphogen, evidencing the ability of morphogens to inhibit glial scar formation and stimulate nerve regeneration.

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Example 12. In Vitro Model for Evaluating Morphogen Species Transport Across the Blood-Brain Barrier.

- 5 Described below is an in vitro method for evaluating the facility with which selected morphogen species likely will pass across the blood-brain barrier. A detailed description of the model and protocol are provided by Audus et al. (1987) Ann. N.Y. Acad. Sci. 507:9-18, the disclosure of which is
- 10 incorporated herein by reference.

Briefly, microvessel endothelial cells are isolated from the cerebral gray matter of fresh bovine brains.

15 Brains are obtained from a local slaughter house and transported to the laboratory in ice cold minimum essential medium (MEM) with antibiotics. Under sterile conditions the large surface blood vessels and meninges are removed using standard dissection procedures. The

20 cortical gray matter is removed by aspiration, then minced into cubes of about 1mm. The minced gray matter then is incubated with 0.5% dispase (BMB, Indianapolis, IN) for 3 hours at 37° C in a shaking water bath. Following the 3 hour digestion, the mixture is

25 concentrated by centrifugation (1000 x g for 10 min.), then resuspended in 13% dextran and centrifuged for 10 min. at 5800 x g. Supernatant fat, cell debris and myelin are discarded and the crude microvessel pellet resuspended in 1 mg/ml collagenase/dispase and

30 incubated in a shaking water bath for 5 hours at 37° C. After the 5-hour digestion, the microvessel suspension is applied to a pre-established 50% Percoll gradient and centrifuged for 10 min at 1000 x g. The band containing purified endothelial cells (second band from

35 the top of the gradient) is removed and washed two

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times with culture medium (e.g., 50% MEM/50% F-12 nutrient mix). The cells are frozen (-80° C.) in medium containing 20% DMSO and 10% horse serum for later use.

5

After isolation, approximately 5×10^5 cells/cm² are plated on culture dishes or 5-12 m μ pore size polycarbonate filters that are coated with rat collagen and fibronectin. 10-12 days after seeding the cells, 10 cell monolayers are inspected for confluence by microscopy.

Characterization of the morphological, histochemical and biochemical properties of these cells 15 has shown that these cells possess many of the salient features of the blood-brain barrier. These features include: tight intercellular junctions, lack of membrane fenestrations, low levels of pinocytotic activity, and the presence of gamma-glutamyl 20 transpeptidase, alkaline phosphatase, and Factor VIII antigen activities.

The cultured cells can be used in a wide variety of experiments where a model for polarized binding or 25 transport is required. By plating the cells in multi-well plates, receptor and non-receptor binding of both large and small molecules can be conducted. In order to conduct transendothelial cell flux measurements, the cells are grown on porous 30 polycarbonate membrane filters (e.g., from Nucleopore, Pleasanton, CA). Large pore size filters (5-12 m μ) are

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used to avoid the possibility of the filter becoming the rate-limiting barrier to molecular flux. The use of these large-pore filters does not permit cell growth under the filter and allows visual inspection of the
5 cell monolayer.

Once the cells reach confluence, they are placed in a side-by-side diffusion cell apparatus (e.g., from Crown Glass, Sommerville, NJ). For flux measurements,
10 the donor chamber of the diffusion cell is pulsed with a test substance, then at various times following the pulse, an aliquot is removed from the receiver chamber for analysis. Radioactive or fluorescently-labelled substances permit reliable quantitation of molecular
15 flux. Monolayer integrity is simultaneously measured by the addition of a non-transportable test substance such as sucrose or inulin and replicates of at least 4 determinations are measured in order to ensure statistical significance.
20

Example 13. Screening Assay for Candidate Compounds which Alter Endogenous Morphogen Levels

Candidate compound(s) which may be administered to
25 affect the level of a given morphogen may be found using the following screening assay, in which the level of morphogen production by a cell type which produces measurable levels of the morphogen is determined with and without incubating the cell in culture with the
30 compound, in order to assess the effects of the compound on the cell. This can be accomplished by detection of the morphogen either at the protein or RNA level. A more detailed description also may be found in international application US92/07359 (WO92/05172).

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13.1 Growth of Cells in Culture

Cell cultures of kidney, adrenals, urinary bladder, brain, or other organs, may be prepared as described widely in the literature. For example, kidneys may be explanted from neonatal or new born or young or adult rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues. Primary tissue cultures and established cell lines, also derived from kidney, adrenals, urinary, bladder, brain, mammary, or other tissues may be established in multiwell plates (6 well or 24 well) according to conventional cell culture techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells may be cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or other growth factors).

Samples for testing the level of morphogen production includes culture supernatants or cell lysates, collected periodically and evaluated for OP-1 production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for RNA analysis. To monitor de novo OP-1 synthesis, some cultures are labeled according to conventional procedures with an ³⁵S-methionine/³⁵S-cysteine mixture for 6-24 hours and then evaluated to OP-1 synthesis by conventional immunoprecipitation methods.

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13.2 Determination of Level of Morphogenic Protein

- In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may
- 5 be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. For example, OP-1 may be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.
- 10 1 μ g/100 μ l of affinity-purified polyclonal rabbit IgG specific for OP-1 is added to each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1%
15 Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C. The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 μ l aliquot of an
20 appropriate dilution of each of the test samples of cell culture supernatant is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100 μ l biotinylated rabbit anti-OP-1 serum (stock solution is about 1 mg/ml and diluted 1:400 in
25 BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20.
100 μ l strepavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in
30 BSB containing 0.1% Tween 20 before use) is added to each well and incubated at 37°C for 30 min. The plates are washed four times with 0.5M Tris buffered Saline

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(TBS), pH 7.2. 50 μ l substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) is added to each well incubated at room temperature for 15 min. Then, 50 μ l amplifier (from the same 5 amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50 μ l 0.3 M sulphuric acid. The OD at 490 nm of the solution in each well is recorded. To quantitate OP-1 in culture media, a OP-1 10 standard curve is performed in parallel with the test samples.

Polyclonal antibody may be prepared as follows. Each rabbit is given a primary immunization of 100 15 ug/500 μ l E. coli produced OP-1 monomer (amino acids 328-431 in SEQ ID NO:5) in 0.1% SDS mixed with 500 μ l Complete Freund's Adjuvant. The antigen is injected subcutaneously at multiple sites on the back and flanks 20 of the animal. The rabbit is boosted after a month in the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against 25 OP-1 is detected in the serum using an ELISA assay. Then, the rabbit is boosted monthly with 100 μ g of antigen and bled (15 ml per bleed) at days seven and ten after boosting.

Monoclonal antibody specific for a given morphogen 30 may be prepared as follows. A mouse is given two injections of E. coli produced OP-1 monomer. The first injection contains 100 μ g of OP-1 in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50 μ g of OP-1 in incomplete adjuvant 35 and is given intraperitoneally. The mouse then

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receives a total of 230 μ g of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, both mice are boosted

- 5 intraperitoneally with 100 μ g of OP-1 (307-431) and 30 μ g of the N-terminal peptide ($\text{Ser}_{293}\text{-Asn}_{309}\text{-Cys}$) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days
- 10 (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as
- 15 antigen. The cell fusion and monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

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SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

- (i) APPLICANT:
(A) NAME: CREATIVE BIOMOLECULES, INC.
(B) STREET: 35 SOUTH STREET
(C) CITY: HOPKINTON
10 (D) STATE: MASSACHUSETTS
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 01748
(G) TELEPHONE: 1-508-435-9001
(H) TELEFAX: 1-508-435-0454
15 (I) TELEX:

(ii) TITLE OF INVENTION: MORPHOGEN-INDUCED NERVE REGENERATION AND REPAIR

20 (iii) NUMBER OF SEQUENCES: 33

- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: CREATIVE BIOMOLECULES, INC.
(B) STREET: 35 SOUTH STREET
25 (C) CITY: HOPKINTON
(D) STATE: MASSACHUSETTS
(E) COUNTRY: USA
(F) ZIP: 01748

30 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

- 35 (vi) ATTORNEY/AGENT INFORMATION:
(A) NAME: KELLEY, ROBIN D.
(B) REGISTRATION NUMBER: 34,637
(C) REFERENCE/DOCKET NUMBER: CRP-070

40 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/248-7000
(B) TELEFAX: 617/248-7100

45 (2) INFORMATION FOR SEQ ID NO:1:

- 50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

5 (ix) FEATURE:

- (A) NAME/KEY: Protein
(B) LOCATION: 1..97
(D) OTHER INFORMATION: /label= GENERIC-SEQ1
/note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
ONE OF THE 20 NATURALLY-OCCURRING L-ISOMER, A-AMINO
ACIDS, OR A DERIVATIVE THEREOF."

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15 Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
20 25 30

20 Xaa
35 40 45

25 Xaa Cys Cys Xaa Xaa
50 55 60

Xaa
65 70 75 80

30 Xaa Cys Xaa Cys
85 90 95

Xaa

35 (2) INFORMATION FOR SEQ ID NO:2:

40 (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: protein

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(ix) FEATURE:

- (A) NAME/KEY: Protein
(B) LOCATION: 1..97
(D) OTHER INFORMATION: /label= GENERIC-SEQ2
5 /note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
ONE OF THE 20 NATURALLY OCCURRING L-ISOMER A-AMINO
ACIDS, OR A DERIVATIVE THEREOF."

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa
1 5 10 15

15 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Cys Xaa
35 40 45

20 Xaa Cys Cys Xaa Xaa
50 55 60

25 Xaa
65 70 75 80

Xaa Cys Xaa Cys
85 90 95

30 Xaa

(2) INFORMATION FOR SEQ ID NO:3:

- 35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

45 (ix) FEATURE:

- (A) NAME/KEY: Protein
(B) LOCATION: 1..97
(D) OTHER INFORMATION: /label= GENERIC-SEQ3
50 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION."

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Ala
1 5 10 15

5

Pro Xaa Gly Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
20 25 30

10

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Leu
35 40 45

Xaa Cys Cys Xaa Pro
50 55 60

15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

20

Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Gly Cys
85 90 95

Xaa

(2) INFORMATION FOR SEQ ID NO:4:

25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: protein

35

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= GENERIC-SEQ4
/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION."

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

45

Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
1 5 10 15

50

Xaa Trp Xaa Xaa Ala Pro Xaa Gly Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
20 25 30

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Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
35 40 45

5 Xaa Xaa Xaa Xaa Leu Xaa Xaa

Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
65 70 75 80

10 Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
85 90 95

Xaa Xaa Cys Gly Cys Xaa
100

(2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: HIPPOCAMPUS

30 (ix) FEATURE:

- (A) NAME/KEY: Protein
(B) LOCATION: 1..139
(D) OTHER INFORMATION: /label= hOP1-MATURE

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
 1 5 10 15

40 Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser
20 25 30

Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
 45 35 40 45

Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
50 55 60

50 Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
 65 70 75 80

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	Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro		
	85	90	95
5	Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile		
	100	105	110
	Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr		
	115	120	125
10	Arg Asn Met Val Val Arg Ala Cys Gly Cys His		
	130	135	

(2) INFORMATION FOR SEQ ID NO:6:

15	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 139 amino acids	
	(B) TYPE: amino acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
20	(ii) MOLECULE TYPE: protein	
	(vi) ORIGINAL SOURCE:	
	(A) ORGANISM: MURIDAE	
25	(F) TISSUE TYPE: EMBRYO	
	(ix) FEATURE:	
	(A) NAME/KEY: Protein	
	(B) LOCATION: 1..139	
30	(D) OTHER INFORMATION: /label= MOP1-MATURE	

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

35	Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys			
	1	5	10	15
	Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser			
	20	25	30	
40	Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg			
	35	40	45	
	Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala			
45	50	55	60	
	Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn			
	65	70	75	80
50	Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro			
	85	90	95	

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Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
100 105 110

5 Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
115 120 125

Arg Asn Met Val Val Arg Ala Cys Gly Cys His
130 135

10 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: HIPPOCAMPUS

25 (ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..139
- (D) OTHER INFORMATION: /label= HOP2-MATURE

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu
1 5 10 15

35 Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser
20 25 30

His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln
35 40 45

40 Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
50 55 60

45 Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn
65 70 75 80

Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
85 90 95

50 Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
100 105 110

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Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
115 120 125

5 Arg Asn Met Val Val Lys Ala Cys Gly Cys His
130 135

(2) INFORMATION FOR SEQ ID NO:8:

- 10 (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (11) MOLECULE TYPE: protein

- 20 (vi) ORIGINAL SOURCE:
(A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO
(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..139
(D) OTHER INFORMATION: /label= MOP2-MATURE

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

30 Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu
1 5 10 15

Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser
20 25 30

35 Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg
35 40 45

Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
50 55 60

40 Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn
65 70 75 80

45 Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
85 90 95

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Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
100 105 110

5 Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
115 120 125

Arg Asn Met Val Val Lys Ala Cys Gly Cys His
130 135

10 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids
- (B) TYPE: amino acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: bovinae

(ix) FEATURE:

- (A) NAME/KEY: Protein
- 25 (B) LOCATION: 1..101
- (D) OTHER INFORMATION: /label= CBMP-2A-FX

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
1 5 10 15

35 Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly
20 25 30

Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
35 40 45

40 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala
50 55 60

45 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
65 70 75 80

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Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu
85 90 95

5 Gly Cys Gly Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:10:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

20 (vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: hippocampus

25 (ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..101
(D) OTHER INFORMATION: /label= CBMP-2B-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

30 Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
1 5 10 15

Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly
20 25 30

35 Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
35 40 45

Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala
50 55 60

40 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
65 70 75 80

45 Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu
85 90 95

Gly Cys Gly Cys Arg
100

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(2) INFORMATION FOR SEQ ID NO:11:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

15 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: DROSOPHILA MELANOGLASTER

20 (ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..101
- (D) OTHER INFORMATION: /label= DPP-FX

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp
1 5 10 15

30 Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly
20 25 30

Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala
35 40 45

35 Val Val Gln Thr Leu Val Asn Asn Asn Pro Gly Lys Val Pro Lys
50 55 60

40 Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu
65 70 75 80

45 Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val
85 90 95

50 40 Val Gly Cys Gly Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:12:

45 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: XENOPUS

5

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VGL-FX

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln
15 1 5 10 15

Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly
20 20 25 30

20 Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala
35 35 40 45

Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu
25 50 55 60

Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr
25 65 70 75 80

30 Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val
85 85 90 95

Asp Glu Cys Gly Cys Arg
100

35 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

40

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE

50

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VGR-1-FX

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

5 Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Val Gly Trp Gln
 1 5 10 15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
10 20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
15 35 40 45

Ile Val Gln Thr Leu Val His Val Met Asn Pro Glu Tyr Val Pro Lys
20 50 55 60

Pro Cys Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val Leu Tyr Phe
25 65 70 75 80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
30 85 90 95

Arg Ala Cys Gly Cys His
20 100

(2) INFORMATION FOR SEQ ID NO:14:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 106 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 30 (ii) MOLECULE TYPE: protein
- 35 (iii) HYPOTHETICAL: NO
- 40 (iv) ANTI-SENSE: NO
- 45 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
 (F) TISSUE TYPE: brain
- 50 (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..106
 (D) OTHER INFORMATION: /note= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

50 Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His
 1 5 10 15

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Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly
20 25 30

Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala
5 35 40 45

Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly
50 55 60

10 Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser
65 70 75 80

Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu
85 90 95

15 Asp Met Val Val Asp Glu Cys Gly Cys Arg
100 105

(2) INFORMATION FOR SEQ ID NO:15:

- 20 (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Xaa Xaa Xaa Xaa
1 5

35 (2) INFORMATION FOR SEQ ID NO:16:

- 40 (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- 50 (vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS

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(ix) FEATURE:

- (A) NAME/KEY: CDS
 - (B) LOCATION: 49..1341
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "OP1"
/evidence= EXPERIMENTAL
/standard name= "OP1"

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCAGC ATG CAC GTG
 Met His Val
 15

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA
 Arg Ser Leu Arg Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
 5 10 15

20

CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC
 Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
 20 25 30 35

25

GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG
 Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
 40 45 50

30

CCG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC
 Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
 55 60 65

35

CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG
 Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
 70 75 80

40

CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC
 Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Pro Gly
 85 90 95

45

GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC
 Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
 100 105 110 115

50

CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC
 Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp
 120 125 130

55

ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC
 Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe
 135 140 145

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	CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile 150 155 160	537
5	CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp 165 170 175	585
10	TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr 180 185 190 195	633
15	CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu 200 205 210	681
20	GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp 215 220 225	729
25	ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu 230 235 240	777
30	GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro 245 250 255	825
35	AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro 260 265 270 275	873
40	TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile 280 285 290	921
45	CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro 295 300 305	969
50	AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser 310 315 320	1017
	AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe 325 330 335	1065
	CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala 340 345 350 355	1113

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	GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met 360 365 370	1161
5	AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn 375 380 385	1209
10	CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala 390 395 400	1257
15	ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys 405 410 415	1305
20	TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His 420 425 430	1351
25	GAGAATTCAAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG	1411
30	TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTT CAGTGGCAGC ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGAAAACC TAGCAGGAAA AAAAAAACAAC GCATAAAGAA AAATGGCCGG GCCAGGTCAAT TGGCTGGAA GTCTCAGCCA TGCACGGACT CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG GGCGTGGCAA GGGGTGGCA CATTGGTGTG TGTGCGAAAG GAAAATTGAC CCCGAAGTTC	1471
35	CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A	1531
		1591
		1651
		1711
		1771
		1822

(2) INFORMATION FOR SEQ ID NO:17:

- 40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 431 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: protein
- (x1) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
50 1 5 10 15

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Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
20 25 30

5 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
35 40 45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
50 55 60

10 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65 70 75 80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly
85 90 95

15 Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
100 105 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
20 115 120 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
130 135 140

25 Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145 150 155 160

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
165 170 175

30 Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
180 185 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
35 195 200 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220

40 Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225 230 235 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255

45 Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
50 275 280 285

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	Arg	Ser	Ile	Arg	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser
	290						295					300				
	Lys	Thr	Pro	Lys	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu
5	305					310					315				320	
	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr
					325					330				335		
10	Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu
					340				345					350		
	Gly	Tyr	Ala	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn
					355				360					365		
15	Ser	Tyr	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His
					370			375					380			
20	Phe	Ile	Asn	Pro	Glu	Thr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
					385		390				395				400	
	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe	Asp	Asp	Ser	Ser	Asn	Val	Ile
					405					410				415		
25	Leu	Lys	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala	Cys	Gly	Cys	His	
					420					425				430		

(2) INFORMATION FOR SEQ ID NO:18:

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

	CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG	60
5	CGGCGCGGGC CCGGTGCCCG GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC Met His Val Arg	115
	1	
10	TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro 5 10 15 20	163
15	CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu 25 30 35	211
20	GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg 40 45 50	259
25	GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro 55 60 65	307
30	CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu 70 75 80	355
35	GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG Asp Leu Tyr Asn Ala Met Ala Val Glu Ser Gly Pro Asp Gly Gln 85 90 95 100	403
40	GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro 105 110 115	451
45	TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val 120 125 130	499
50	ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro 135 140 145	547
55	CGA TAC CAC CAT CGG GAG TTC CCG TTT GAT CTT TCC AAG ATC CCC GAG Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu 150 155 160	595
60	GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile 165 170 175 180	643

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	CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val 185	190	195	691
5	CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser 200	205	210	739
10	CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr 215	220	225	787
15	GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CCG CAC AAC CTG GGC TTA Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu 230	235	240	835
20	CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu 245	250	255	883
25	GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met 265	270	275	931
30	GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser 280	285	290	979
35	ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn 295	300	305	1027
40	CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp 310	315	320	1075
45	CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp 325	330	335	1123
50	CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr 345	350	355	1171
	TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala 360	365	370	1219
	ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp 375	380	385	1267

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	ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT	1315
	Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser	
	390 395 400	
5	GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC GAC CTG AAG AAG TAC AGA	1363
	Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu Lys Lys Tyr Arg	
	405 410 415 420	
10	AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTTTCC TGAGACCCTG	1413
	Asn Met Val Val Arg Ala Cys Gly Cys His	
	425 430	
	ACCTTGGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
15	CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCCTGAGCC TTCCCTCACCC TCCCAACCGG	1533
	AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCCTCT	
20	GGCACGTGAC GGACAAGATC CTACCAAGCTA CCACAGCAAA CGCCTAACAGAG CAGGAAAAAT	1653
	GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCCTGGC GCTCTGAGTC TTTGAGGAGT	
	AATCGCAAGC CTCGTTCAAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCCTGGCG	1773
25	TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT	1833
	GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTG	1873

30 (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

40	Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala	
	1 5 10 15	
45	Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser	
	20 25 30	
	Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser	
	35 40 45	
50	Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu	
	50 55 60	

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	Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro			
65	65	70	75	80
	Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly			
5	85	90	95	
	Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr			
	100	105	110	
10	Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp			
	115	120	125	
	Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu			
	130	135	140	
15	Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser			
	145	150	155	160
	Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr			
20	165	170	175	
	Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr			
	180	185	190	
25	Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe			
	195	200	205	
	Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val			
	210	215	220	
30	Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His			
	225	230	235	240
	Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile			
35	245	250	255	
	Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys			
	260	265	270	
40	Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg			
	275	280	285	
	Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys			
	290	295	300	
45	Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn			
	305	310	315	320
	Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val			
50	325	330	335	

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	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly
					340				345				350			
5	Tyr	Ala	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser
					355				360			365				
	Tyr	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Phe
		370				375					380					
10	Ile	Asn	Pro	Asp	Thr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	Leu
		385				390		395				395		400		
	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe	Asp	Asp	Ser	Ser	Asn	Val	Asp	Leu
						405			410				415			
15	Lys	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala	Cys	Gly	Cys	Cys	His	
					420			425					430			

(2) INFORMATION FOR SEQ ID NO:20:

- 20 (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1723 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: cDNA
- 30 (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
 - (F) TISSUE TYPE: HIPPOCAMPUS
- 35 (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 490..1696
 - (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "hOP2-PP"
/note= "hOP2 (cDNA)"

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCGCCGGCA	GAGCAGGAGT	GGCTGGAGGA	GCTGTGGTTG	GAGCAGGAGG	TGGCACGGCA	60	
45	GGGCTGGAGG	GCTCCCTATG	AGTGGCGGAG	ACGGCCCAGG	AGGCGCTGGA	GCAACAGCTC	120
	CCACACCGCA	CCAAGCGGTG	GCTGCAGGAG	CTCGCCCCATC	GCCCCCTGCGC	TGCTCGGACC	180
50	GCGGCCACAG	CCGGACTGGC	GGGTACGGCG	GCGACAGAGG	CATTGGCCGA	GAGTCCCAGT	240

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	CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG	300
	GACAGGTGTC GCGCGGCCGG GCTCCAGGGA CGCGCCCTGA GGCGGGCTGC CGCCCCGTCC	360
5	CGCCCCGCC CGCCGCCCGC CGCCCCCGA GCCCAGCCTC CTTGCCGTG GGGCGTCCCC	420
	AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC	480
10	CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu	528
	1 5 10	
15	GCG CTA TGC GCG CTG GGC GGG GGC CCC GGC CTG CGA CCC CCG CCC Ala Leu Cys Ala Leu Gly Gly Pro Gly Leu Arg Pro Pro Pro	576
	15 20 25	
	GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln	624
	30 35 40 45	
20	CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg	672
	50 55 60	
25	GCG CCA CCC GCC TCC CCG CTG CCC GCG TCC GCG CCG CTC TTC ATG Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met	720
	65 70 75	
30	CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala	768
	80 85 90	
35	CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val	816
	95 100 105	
	AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp	864
	110 115 120 125	
40	AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val	912
	130 135 140	
45	ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu	960
	145 150 155	
50	AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser	1008
	160 165 170	

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	AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT	1056
	Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala	
175	180	185
5	GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC	1104
	Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys	
190	195	200
10	TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG	1152
	Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu	
	210	215
	220	
15	ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT	1200
	Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly	
	225	230
	235	
	CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG	1248
	Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg	
	240	245
	250	
20	GCC AGT CCG AGT CCC ATC CGC ACC CCT CCG GCA GTG AGG CCA CTG AGG	1296
	Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg	
	255	260
	265	
25	AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC	1344
	Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu	
	270	275
	280	285
30	CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC	1392
	Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys	
	290	295
	300	
35	CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC	1440
	Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp	
	305	310
	315	
	TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG	1488
	Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu	
	320	325
	330	
40	TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC	1536
	Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile	
	335	340
	345	
45	CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG	1584
	Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala	
	350	355
	360	365

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TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp 370 375 380	1632
5 AGC AGC AAC AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC AAG Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys 385 390 395	1680
GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG 10 Ala Cys Gly Cys His 400	1723

(2) INFORMATION FOR SEQ ID NO:21:

15 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 402 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear	
20 (ii) MOLECULE TYPE: protein	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:	
25 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys 1 5 10 15	
Ala Leu Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro 20 25 30	
30 Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile 35 40 45	
35 Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro 50 55 60	
Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu 65 70 75 80	
40 Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu 85 90 95	
Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val 100 105 110	
45 Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe 115 120 125	
50 Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala 130 135 140	

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Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
145 150 155 160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
5 165 170 175

Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
180 185 190

10 Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
195 200 205

Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
15 210 215 220

Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
225 230 235 240

Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
20 245 250 255

Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
260 265 270

25 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
275 280 285

Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
30 290 295 300

Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
305 310 315 320

Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
35 325 330 335

Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
340 345 350

40 Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
355 360 365

Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
45 370 375 380

Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly
385 390 395 400

Cys His
50

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(2) INFORMATION FOR SEQ ID NO:22:

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	AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu 105 110 115	449
5	CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT GGG Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly 120 125 130 135	497
10	GAG GCT GTC ACA GCT GCT GAG TTC CCG ATC TAC AAA GAA CCC AGC ACC Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser Thr 140 145 150	545
15	CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC CAA His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val Gln 155 160 165	593
20	GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr 170 175 180	641
25	CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA GCC Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala Ala 185 190 195	689
30	AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC CTC Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg Leu 200 205 210 215	737
35	TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT GGT Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala Gly 220 225 230	785
40	CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA ACC Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val Thr 235 240 245	833
45	TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG AGA Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala Arg 250 255 260	881
50	CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC CCC Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His Pro 265 270 275	929
	AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC AGA Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly Arg 280 285 290 295	977
	GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT GAC CTT GGC Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly 300 305 310	1025

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	TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC TGT Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys 315	320	325	1073
5	GAG GGG GAG TGT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn 330	335	340	1121
10	CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val 345	350	355	1169
15	CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu 360	365	370	1217
20	TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met 380	385	390	1265
25	GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC TGCTTCTACT Val Val Lys Ala Cys Gly Cys His 395			1319
30	ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCTGCTA AAATTCTGGT CTTCCCCAGT TCCTCTGTCC TTCATGGGT TTGGGGCTA TCACCCCGCC CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGCCATC CTCAGCCAC			1379
35	AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTCAAACTAGAT GATCTGGGCT CTCTGCACCA TTCAATTGTGG CAGTTGGAC ATTTTTAGGT ATAACAGACA CATAACACTTA GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAACAGAG CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAAATCT CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAAC			1439
40				1499
45	GGAATTG			1559
				1619
				1679
				1739
				1799
				1859
				1919
				1926

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(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1 5 10 15

15 Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
20 25 30

Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu
35 40 45

20 Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala
50 55 60

His Ala Met Thr Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu
85 90 95

30 Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp
100 105 110

Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp
115 120 125

35 Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg
130 135 140

40 Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile
 40 145 150 155 160

Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu
165 170 175

45 Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu
180 185 190

Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His
195 200 205

50 Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser
210 215 220

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Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser
225 230 235 240

5 Arg Gln Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val
245 250 255

Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys
260 265 270

10 Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp
275 280 285

Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr
290 295 300

15 Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln
305 310 315 320

Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp
20 325 330 335

Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His
340 345 350

25 Leu Met Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys
355 360 365

Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile
370 375 380

30 Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
385 390 395

(2) INFORMATION FOR SEQ ID NO:24:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1368 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1368

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

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	ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC	48
	Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser	
	1 5 10 15	
5	CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG	96
	Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro	
	20 25 30	
10	GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC	144
	Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp	
	35 40 45	
15	CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC	192
	Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val	
	50 55 60	
	TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC	240
	Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His	
	65 70 75 80	
20	CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG	288
	Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu	
	85 90 95	
25	CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG	336
	Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln	
	100 105 110	
30	GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC	384
	Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala	
	115 120 125	
35	GAC CTC GAG GAT GAG GGC GAG CAG CAG AAG AAC TTC ATC ACC GAC	432
	Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp	
	130 135 140	
	CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG	480
	Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu	
	145 150 155 160	
40	AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT	528
	Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg	
	165 170 175	
45	CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG	576
	Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val	
	180 185 190	
50	ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG	624
	Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu	
	195 200 205	

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	ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly 210 215 220	672
5	ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr 225 230 235 240	720
10	GGG GAC TAC GTG GGC TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His 245 250 255	768
15	GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala 260 265 270	816
20	CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly 275 280 285	864
25	CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly 290 295 300	912
30	TTC TTC CGC GGA CCG GAG CTG ATC AAG GCG ACG GCC CAC AGC AGC CAC Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His 305 310 315 320	960
35	CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser 325 330 335	1008
40	GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg 340 345 350	1056
45	AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp 355 360 365	1104
50	CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser 370 375 380	1152
55	GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His 385 390 395 400	1200
60	GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro 405 410 415	1248

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AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC	1296
Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr	
420	425
430	
5 CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT	1344
His Leu Asn Asp Glu Asn Val Asn Leu Lys Tyr Arg Asn Met Ile	
435	440
445	
10 GTG AAA TCC TGC GGG TGC CAT TGA	1368
Val Lys Ser Cys Gly Cys His	
450	455

(2) INFORMATION FOR SEQ ID NO:25:

15 (i) SEQUENCE CHARACTERISTICS:			
	(A) LENGTH: 455 amino acids		
	(B) TYPE: amino acid		
	(D) TOPOLOGY: linear		
20 (ii) MOLECULE TYPE: protein			
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:		
25 Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser			
1	5	10	15
Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro			
20	25	30	
30 Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp			
35	40	45	
35 Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val			
50	55	60	
65 Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His			
70	75	80	
40 Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu			
85	90	95	
100 Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln			
105	110		
115 Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala			
120	125		
130 Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp			
135	140		

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	Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu		
145	150	155	
	Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg		
5	165	170	175
	Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val		
	180	185	190
10	Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu		
	195	200	205
	Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly		
	210	215	220
15	Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr		
	225	230	235
	Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His		
20	245	250	255
	Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala		
	260	265	270
25	His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly		
	275	280	285
	Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly		
	290	295	300
30	Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His		
	305	310	315
	320		
35	His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser		
	325	330	335
	Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg		
	340	345	350
40	Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp		
	355	360	365
	His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser		
	370	375	380
45	Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His		
	385	390	395
	400		
50	Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro		
	405	410	415

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Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
420 425 430

5 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
435 440 445

Val Lys Ser Cys Gly Cys His
450 455

10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 104 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: protein

25

(ix) FEATURE:

- (A) NAME/KEY: Protein
(B) LOCATION: 1..104
(D) OTHER INFORMATION: /note= "BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

30 Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
1 5 10 15

35 Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly
20 25 30

Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
35 40 45

40 Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
50 55 60

45 Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
65 70 75 80

50 Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
85 90 95

55 Thr Val Glu Ser Cys Ala Cys Arg
100

50

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(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

15 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS

20 (ix) FEATURE:

- 15 (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 (D) OTHER INFORMATION: /note= "BMP5"

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
1 5 10 15

30 Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

35 Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
50 55 60

40 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

45 Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
85 90 95

50 Arg Ser Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:28:

45 (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: protein

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(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

5 (ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /note= "BMP6"

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
1 5 10 15

15 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

20 Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
50 55 60

25 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
85 90 95

30 Arg Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:29:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= OPX

/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION (SECTION II.B.2.)"

50

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1 5 10 15

5 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
20 25 30

10 Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
35 40 45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
50 55 60

15 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65 70 75 80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
20 85 90 95

25 Xaa Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:30:

25 (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
30 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

35 (ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..97
40 (D) OTHER INFORMATION: /label= GENERIC-SEQ5
/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION."

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Xaa
1 5 10 15

50 Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
20 25 30

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

10 Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
85 90 95

Xaa

15

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(iii) MOLECULE TYPE: protein

25

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1., 102

(D) OTHER INFORMATION: /label- GENERIC-SEQ6

/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS AS DEFINED IN THE SPECIFICATION. "

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
 1 5 10 15

40

Xaa Trp Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
20 25 30

Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
35 40 45

50

Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
 65 70 75 80

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Xaa	Xaa	Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Met	Xaa	Val	
					85								90		95

Xaa	Xaa	Cys	Xaa	Cys	Xaa
5					100

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 1247 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: BRAIN

20 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 84..1199
- (D) OTHER INFORMATION: /product= "GDF-1"
- 25 /note= "GDF-1 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

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35	GGC CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC	158
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40	CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC CAG	206
	Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu Gln	
	30 35 40	
45	GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC CGG CCG	254
	Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu Arg Pro	
	45 50 55	
50	GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC CCC CAG GAG	302
	Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Asp Pro Gln Glu	
	60 65 70	

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	75 80 85	
5	TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC ATC GTG CGC CAC ATC Cys His Val Glu Glu Leu Gly Val Ala Gly Asn Ile Val Arg His Ile	398
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10	CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG GAG CCT GTC TCG GCC GCG Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser Glu Pro Val Ser Ala Ala	446
	110 115 120	
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	125 130 135	
20	CCC GCT GAG CGC CCG AGC CGG GCC CGC CTG GAG CTG CGT TTC GCG GCG Pro Ala Glu Arg Pro Ser Arg Ala Arg Leu Glu Leu Arg Phe Ala Ala	542
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25	GCG GCG GCG GCA GCC CCG GAG GGC GGC TGG GAG CTG AGC GTG GCG CAA Ala Ala Ala Ala Pro Glu Gly Gly Trp Glu Leu Ser Val Ala Gln	590
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	190 195 200	
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	270 275 280	

- 152 -

CAC CGC TGG GTC ATC GCG CCG CGC GGC TTC CTG GCC AAC TAC TGC CAG His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln 285 290 295	974
5 GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG GGG CCG CCG Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro 300 305 310	1022
10 GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC GCG GCC GCC CCG Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro 315 320 325	1070
15 GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG CGC CTG TCG CCC ATC Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile 330 335 340 345	1118
20 TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC GTG GTG CTG CGG CAG TAT Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr 350 355 360	1166
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25 CCCGGGCCA ACAATAATG CCGCGTGG	1247

(2) INFORMATION FOR SEQ ID NO:33:

30	(i) SEQUENCE CHARACTERISTICS:
	(A) LENGTH: 372 amino acids
	(B) TYPE: amino acid
	(D) TOPOLOGY: linear
35	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
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	Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro 20 25 30
45	Pro Gly Pro Ala Ala Leu Leu Gln Ala Leu Gly Leu Arg Asp Glu 35 40 45
	Pro Gln Gly Ala Pro Arg Leu Arg Pro Val Pro Pro Val Met Trp Arg 50 55 60
50	Leu Phe Arg Arg Asp Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg 65 70 75 80

- 153 -

Thr Ser Pro Gly Val Thr Leu Gln Pro Cys His Val Glu Glu Leu Gly
85 90 95

5 Val Ala Gly Asn Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr
100 105 110

Arg Ala Ser Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr
115 120 125

10 Val Val Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg
130 135 140

Ala Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu
145 150 155 160

15 Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly Ala
165 170 175

Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu Gly Pro
20 180 185 190

Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg Asn Ala Ser
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25 Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg Pro Arg Ala Pro
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Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu Leu Leu Val Thr Leu
225 230 235 240

30 Asp Pro Arg Leu Cys His Pro Leu Ala Arg Pro Arg Arg Asp Ala Glu
245 250 255

35 Pro Val Leu Gly Gly Pro Gly Gly Ala Cys Arg Ala Arg Arg Leu
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Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro
275 280 285

40 Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val
290 295 300

Ala Leu Ser Gly Ser Gly Pro Pro Ala Leu Asn His Ala Val Leu
305 310 315 320

45

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Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys
325 330 335

5 Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn
340 345 350

Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu
355 360 365

10 Cys Gly Cys Arg
370

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What is claimed is:

1. The use of a morphogen in the manufacture of a pharmaceutical for enhancing survival of neural cells at risk of dying.
- 5
2. A method for enhancing survival of neural cells at risk of dying, the method comprising providing a morphogen to said cells at a concentration and for a time sufficient to enhance survival of said cells.
- 10
3. The invention of claim 1 or 2 wherein said cells are at risk of dying due to chemical or mechanical trauma to nerve tissue comprising said cells.
- 15
4. The invention of claim 3 wherein said trauma comprises a transected nerve.
- 20
5. The invention of claim 3 wherein said morphogen is provided to said cells prior to said trauma.
6. The invention of claim 3 wherein said trauma results in demyelination of said cells.
- 25
7. The invention of claim 3 wherein said trauma results from exposure of said cells to a cellular toxin.
- 30
8. The invention of claim 7 wherein said toxin comprises ethanol.

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9. The invention of claim 1 or 2 wherein said cells are at risk of dying due to a neuropathy.
10. The invention of claim 9 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.
11. The invention of claim 10 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis or Alzheimer's disease.
12. The invention of claim 1 or 2 wherein said cells are at risk of dying due a neoplastic lesion associated with nerve tissue comprising said cells.
13. The invention of claim 12 wherein said lesion results from a neoplasm comprising cells of neuronal origin.
14. The invention of claim 13 wherein said neoplasm comprises a neuroblastoma or a retinoblastoma.
15. The invention of claim 12 wherein said lesion results from a neoplasm comprising glial cells.
16. The invention of claim 1 or 2 wherein said neural cells at risk of dying comprise part of the central nervous system.
17. The invention of claim 16 wherein said cells comprise striatal basal ganglia neurons.

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18. The invention of claim 16 wherein said cells comprise neurons of the substantia nigra.
19. The invention of claim 1 or 2 wherein said cells at 5 risk of dying comprise part of the peripheral nervous system.
20. The invention of claim 1 or 2 wherein said morphogen stimulates cell adhesion molecule production in said cells. 10
21. The invention of claim 20 wherein said cell adhesion molecule is a nerve cell adhesion molecule. 15
22. The invention of claim 21 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.
23. The invention of claim 1 or 2 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 25 60A(fx).
24. The invention of claim 23 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, 30 Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).

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25. The invention of claim 24 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

5

26. The invention of claim 25 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

10

27. The invention of claim 22 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

15

28. A method for enhancing the survival of neural cells at risk of dying in a mammal, the method comprising the step of administering to said mammal an effective amount of an agent capable of stimulating production of an endogenous morphogen.

20

29. The method of claim 28 wherein said agent stimulates production of an endogenous morphogen in the tissue comprising said neural cells.

25

30. A method for maintaining a neural pathway in a mammal, comprising:
providing a morphogen to the neurons defining said pathway at a concentration and for a time sufficient to maintain said pathway.

30

31. The method of claim 30 wherein said morphogen is provided prior to injury to said pathway.

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32. The method of claim 30 wherein said morphogen is sufficient to stimulate repair of a damaged neural pathway.

5 33. The method of claim 32 wherein said damaged neural pathway results from mechanical or chemical trauma to said pathway.

10 34. The method of claim 33 wherein said trauma comprises a severed nerve.

15 35. The method of claim 33 wherein said trauma comprises demyelination of the neurons defining said pathway.

36. The method of claim 33 wherein said trauma results from exposure of the cells defining said pathway to a cellular toxin.

20 37. The method of claim 36 wherein said toxin comprises ethanol.

25 38. The method of claim 30 wherein said damaged neural pathway results from a neuropathy of the cells defining said pathway.

39. The method of claim 38 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.

30 40. The method of claim 39 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis, or Alzheimer's disease.

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41. The method of claim 38 wherein said neuropathy comprises axonal degeneration.
42. The method of claim 38 wherein said neuropathy comprises a demyelinating neuropathy.
5
43. The method of claim 30 wherein said damaged neural pathway results from a neoplastic lesion.
- 10 44. The method of claim 43 wherein said neoplastic lesion is caused by a neuroblastoma or a glioma.
45. The method of claim 30 wherein said morphogen stimulates cell adhesion molecule production in a
15 cell defining said pathway.
46. The method of claim 45 wherein said cell adhesion molecule is a nerve cell adhesion molecule.
- 20 47. The method of claim 46 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.
48. The method of claim 30 or 45 wherein said morphogen
25 comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

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49. The method of claim 48 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2,
5 Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).
50. The method of claim 49 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence
10 defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
51. The method of claim 50 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence
15 defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
52. The method of claim 51 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.), including
20 allelic and species variants thereof.
53. The invention of claims 1, 2, 30 or 46 wherein said morphogen comprises a polypeptide chain encoded by a nucleic acid that hybridizes under stringent
25 conditions with the DNA sequence defined by nucleotides 1036-1341 of Seq. Id No. 16 or nucleotides 1390-1695 of Seq. ID No. 20.
54. The invention of claims 1, 2, 26, 30, 45 or 51
30 wherein said morphogen comprises a dimeric protein species complexed with a peptide comprising a pro region of a member of the morphogen family, or an allelic, species or other sequence variant thereof.

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55. The invention of claim 54 wherein said dimeric morphogen species is noncovalently complexed with said peptide.

5 56. The invention of claims 54 or 55 wherein said dimeric morphogen species is complexed with two said peptides.

10 57. The invention of claims 54 or 55 wherein said peptide comprises at least the first 18 amino acids of a sequence defining said pro region.

58. The invention of claim 57 wherein said peptide comprises the full length form of said pro region.

15 59. The invention of claims 54 or 55 wherein said peptide comprises a nucleic acid that hybridizes under stringent conditions with a DNA defined by nucleotides 136-192 of Seq. ID No. 16, or
20 nucleotides 157-211 of Seq. ID No. 20.

60. The invention of claims 54 or 55 wherein said complex is further stabilized by exposure to a basic amino acid, a detergent or a carrier protein.

25 61. A method of maintaining a neural pathway in a mammal comprising:
30 administering said mammal an effective amount of an agent capable of stimulating production of an endogenous morphogen in a cell defining said pathway.

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62. A composition for promoting regeneration of a neural pathway at a site of injury in a mammal, comprising:

5 a biocompatible, in vivo bioresorbable carrier suitable for maintaining a protein at a site in vivo, and

10 a morphogen, such that said morphogen, when dispersed in said carrier and provided to said site of injury, is capable of stimulating neural pathway regeneration at said site.

- 15 63. The composition of claim 62 wherein said carrier is structurally sufficient to assist direction of axonal growth.

- 15 64. The composition of claim 63 wherein said carrier comprises a polymeric material.

- 20 65. The composition of claim 63 wherein said carrier comprises laminin or collagen.

66. A device for repairing a break in a neural pathway, the device comprising:

25 a biocompatible tubular casing comprising an exterior and an interior surface and defining a channel through which a neural process may regenerate,

30 said device having a shape and dimension sufficient to span a break in a neural pathway, and having openings adapted to receive the ends of a severed nerve, and

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a morphogen disposed within the channel defined by said tubular casing and accessible to severed nerve ends defining a break in a neural pathway, such that said morphogen stimulates neural pathway regeneration when disposed in said channel and accessible to said nerve ends.

- 5 67. The device of claim 66 wherein said morphogen is disposed in said channel together with a biocompatible, bioresorbable carrier suitable for maintaining a protein at a site in vivo.
- 10 68. The device of claim 67 wherein said carrier comprises sufficient structure to assist direction of axonal growth within said channel.
- 15 69. The device of claim 67 wherein the outer surface of said casing is substantially impermeable.
- 20 70. The device of claim 66 wherein said carrier comprises a polymer.
- 25 71. The device of claim 67 wherein said carrier comprises laminin or collagen.
- 30 72. A method for inducing the redifferentiation of transformed cells of neural origin, the method comprising the step of:
 contacting said transformed cells with a morphogen composition at a concentration and for a time sufficient to induce redifferentiation of said cells to a morphology characteristic of untransformed neuronal cells.

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73. The method of claim 72 wherein said morphology characteristic of untransformed nerve cells includes formation of neurite outgrowths.

5 74. The method of claim 72 wherein said morphology characteristic of untransformed nerve cells includes cell aggregation and cell adhesion.

10 75. The method of claim 72 wherein said morphogen composition induces nerve cell adhesion molecule production in said cells.

15 76. The method of claim 72 wherein said induced nerve cell adhesion molecules include N-CAM-180, N-CAM-140 and N-CAM-120.

77. The method of claim 72 wherein said transformed cells comprise neuroblastoma cells.

20 78. A kit for detecting a neuropathy in a mammal or for evaluating the efficacy of a therapy for treating a neuropathy in a mammal, the kit comprising:
c) means for capturing a cell or body fluid sample obtained from a mammal;
25 b) a binding protein that interacts specifically with a morphogen in said sample so as to form a binding protein-morphogen complex;
c) means for detecting said complex.

30 79. The kit of claim 78 which said binding protein has specificity for an epitope defined by part or all of the pro region of a morphogen.

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80. A method for detecting a neuropathy in a mammal, the method comprising the step of:

detecting fluctuations in the physiological concentration of a morphogen present in the serum or cerebrospinal fluid of said mammal, said fluctuations being indicative of an increase in neuronal cell death.

- 5 81. A method for detecting a neuropathy in a mammal, the method comprising the step of:

detecting fluctuations in the physiological concentration of a morphogen antibody titer present in the serum or cerebrospinal fluid of said mammal, said fluctuations being indicative of an increase

15 in neuronal cell death.

- 20 82. The invention of claims 78, 80 or 81 wherein said neuropathy results from a neurodegenerative disease, nerve demyelination, myelin dysfunction, neuronal neoplasias, or nerve trauma.

- 25 83. A method of stimulating production of cell adhesion molecules in a tissue comprising the step of:

providing a morphogen to said tissue for a time and at a concentration sufficient to induce production of cell adhesion molecules in cells of said tissue.

- 30 84. The method of claim 83 wherein said cell adhesion molecules comprises nerve cell adhesion molecules.

- 35 85. The method of claim 84 wherein said cells comprise neurons.

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86. The method of claim 78, 80 or 81 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

5
87. The method of claim 86 wherein said morphogen comprises an amino acid sequence sharing at least 10 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A (fx).

15
88. The method of claim 87 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

20
89. The method of claim 88 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

25
90. The method of claim 89 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

30
91. The method of claim 78, 80 or 81 wherein said morphogen comprises an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with the sequence defined by nucleotides 1036-1341 of Seq. ID No. 16 or nucleotides 1390-1695 of Seq. ID No. 20.

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92. A composition for enhancing survival of neuronal cells at risk of dying comprising a morphogen in association with a molecule capable of enhancing the transport of said morphogen across the
5 blood-brain barrier.
93. The invention of claims 62 or 67 wherein said carrier comprises brain tissue derived extracellular matrix.

1/3

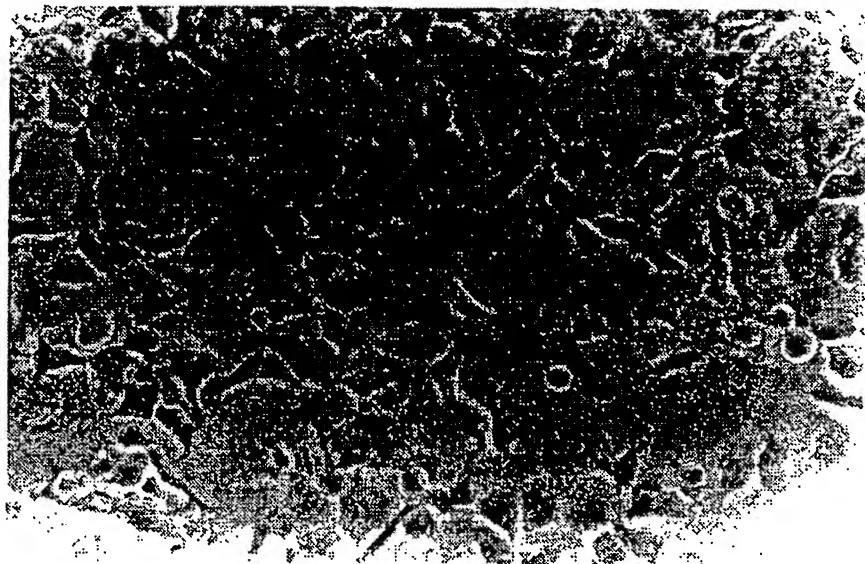


Fig. 1A

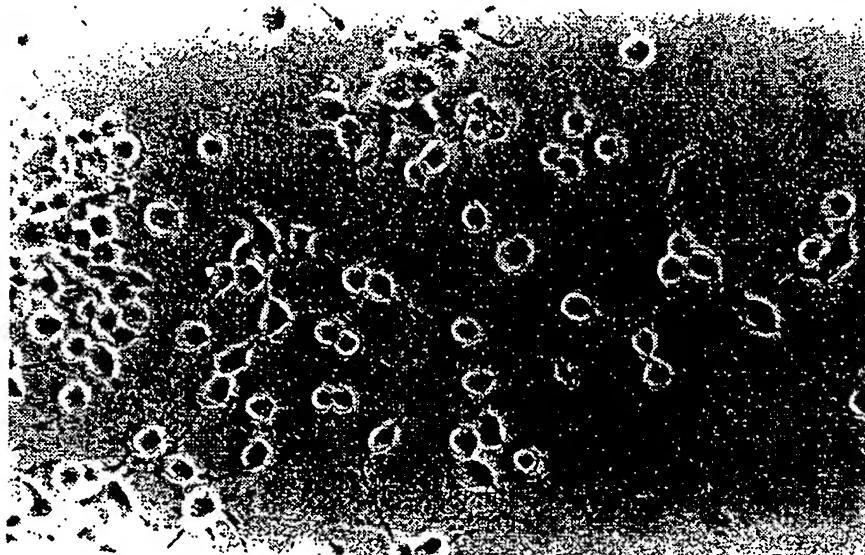


Fig. 1B

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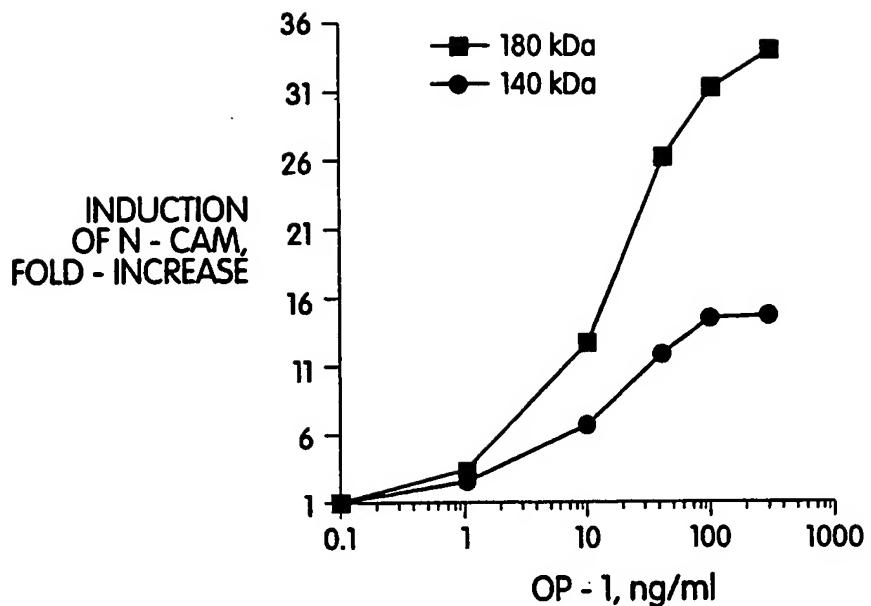


Fig. 2A

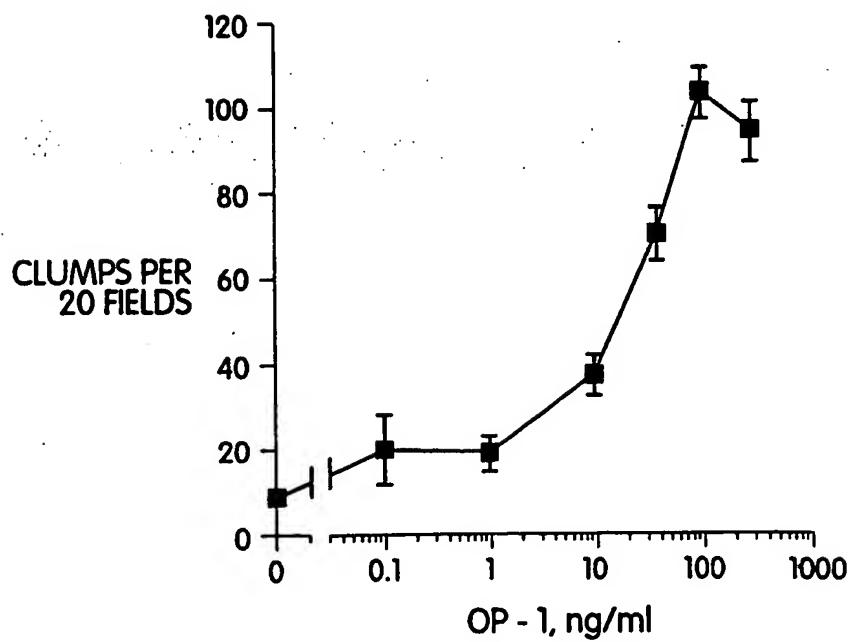


Fig. 3

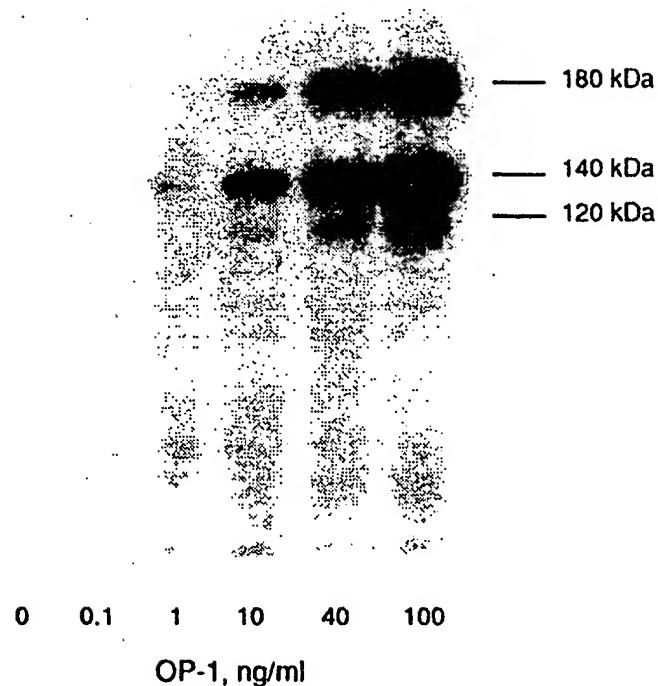


Fig. 2B

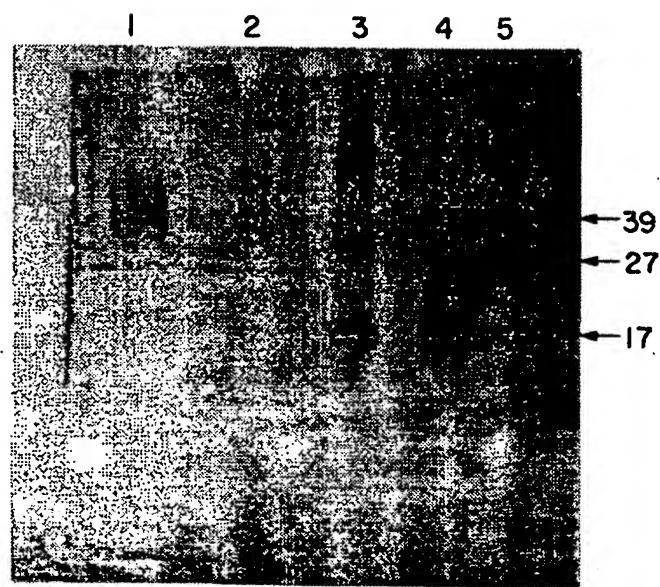


Fig. 4

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 93/07231A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K37/02 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 00382 (CARNEGIE INSTITUTION OF WASHINGTON) 9 January 1992 see page 9, line 15 - page 15, line 29 ---	1-24,78, 79,82, 86,87
X,P	WO,A,92 15323 (CREATIVE BIOMOLECULES, INC.) 17 September 1992 cited in the application see page 6, line 1 - page 26, line 18 ---	1-93
X,P	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89 , November 1992 , WASHINGTON US pages 10326 - 10330 GEORGE PERIDES ET AL. 'INDUCTION OF THE NEURAL CELL ADHESION MOLECULE AND NEURONAL AGGREGATION BY OSTEOGENIC PROTEIN 1' THE WHOLE ARTICLE ---	1,20-27, 53

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *'A' document defining the general state of the art which is not considered to be of particular relevance
- *'E' earlier document but published on or after the international filing date
- *'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *'O' document referring to an oral disclosure, use, exhibition or other means
- *'P' document published prior to the international filing date but later than the priority date claimed

- *'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *'A' document member of the same patent family

1 Date of the actual completion of the international search 8 November 1993	Date of mailing of the international search report 07.12.93
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer REMPP, G

INTERNATIONAL SEARCH REPORT

Inten al Application No

PCT/US 93/07231

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOLOGICAL ABSTRACTS vol. 91 1991, Philadelphia, PA, US; abstract no. 106862, JONES, C. ET AL. 'INVOLVEMENT OF BONE MORPHOGENETIC PROTEIN-4 (BMP-4) AND VGR-1 IN MORPHOGENESIS AND NEUROGENESIS IN THE MOUSE' see abstract & DEVELOPMENT (CAMB) vol. 111, no. 2 , 1991 pages 531 - 542 -----	

INTERNATIONAL SEARCH REPORT**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 2,28-52,61,72-77,80,81,83,85 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple invasions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern'l Application No

PCT/US 93/07231

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9200382	09-01-92	AU-A-	8496491	23-01-92
WO-A-9215323	17-09-92	AU-A-	1754392	06-10-92

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